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**"PROTOZOAL PARASITES OF FRESHWATER FISH".**

**A thesis submitted  
for the degree of Ph.D.  
to the University of London.**

**by**

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**(From the Department of Parasitology,  
London School of Hygiene and Tropical Medicine).**

**January, 1952.**

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## CONTENTS.

1. Acknowledgement. .. .. .	
2. Introduction. .. .. .	1
3. Future field of study and investigation. .. .. .	3
4. Materials and methods. .. .. .	6
5. Historical account of trypanosomes of fish. .. .. .	8

## PART (I)

6. The morphology and life cycle in the host and in culture of <u>Trypanosoma striati</u> , a parasite of an Indian freshwater fish. .. .. .	10
(a) Morphology of forms found in the host. .. .. .	11
(b) Cultivation of <u>T. striati</u> . .. .. .	18
(1) Methods of cultivation. .. .. .	21
(11) Micro isolation technique. .. .. .	23
1. Dilution method. .. .. .	23
2. Capillary pipette method. .. .. .	25
(c) General account of cultural forms and life cycle. .. .. .	26

(d)	Types of parasite in culture. . . . .	41
7.	Effect of temperature in relation to culture. . . . .	45
8.	A study of the life cycle of blood and cultural forms of the trypanosome of the <u>Mirror carp</u> . . . . .	49
(a)	Description of the trypanosome in the blood of the <u>Mirror carp</u> . . . . .	49
(b)	Culture of trypanosome of the <u>Mirror carp</u> . . . . .	54
(c)	General account of the morphology of cultural forms. . . . .	55
(d)	Division... . . . .	60
9.	Comparative study of cultural forms of <u>T. striati</u> and of the trypanosome of the <u>Mirror carp</u> ... . .	62
10.	Differences of cultivation; structure of the organism in fluid and solid media. . . . .	63
11.	A comparison of the cycles of development of <u>T. winchii</u> in artificial culture and <u>T. danilewskyi</u> in the invertebrate host. . . . .	66
12.	An experimental study of <u>T. danilewskyi</u> and its transmission by the leech <u>Hemiclepsis marginata</u> ... . .	67
13.	<u>Trypanoplasma willoughbii</u> n.sp. of the Char ( <u>Salvelinus willoughbii</u> ) and its culture. . . . .	86
14.	Description of flagellata found in the blood of different species of fish. . . . .	103
(a)	<u>Trypanoplasma guernei</u> . . . . .	104

(b)	<u>Trypanoplasma cyprini.</u>	..	..	..	106
(c)	<u>Trypanosoma danilewskyi var batrachi.</u>	..			107
(d)	<u>T. danilewskyi var saccobranhi...</u>	..			112
(e)	<u>Trypanosoma abramidis.</u>	..	..	..	115
(f)	<u>Trypanosoma percae.</u>	..	..	..	119

## PART (II)

### MYXOSPORIDIA.

15.	<u>Myxosporidia.</u>	..	..	..	..	..	122
	(a) <u>Myxobolus clarii.</u>	..	..	..	..		124
	(b) <u>Myxidium scardini</u> n.sp.	..	..	..			131
	(c) <u>Zschokkella cyprini</u> n.sp.	..	..	..			134
	(d) <u>Henneguya psorospermica.</u>	..	..	..			138
	(e) <u>Chloromyxum phoxini</u> n.sp.	..	..	..			140

## PART (III)

### CILIATA.

16.	<u>Ciliata.</u>	..	..	..	..	..	145
	(a) <u>Cyclochaeta domergui.</u>	..	..	..			145
	(b) <u>Ichthyophtherius multifiliis.</u>	..	..				147

17.	<u>Abstract.</u>	..	..	..	..	..	
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18.	<u>References.</u>	..	..	..	..	..	
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Biological Association I have to return sincere thanks  
for helping me by the supply of leeches and many  
available species of freshwater fish from Lake Windermere.

INTRODUCTION.

The present investigation has been devoted to parasitic protozoa of freshwater fish of Great Britain, Europe and India.

In the first half of the nineteenth century the parasitic protozoa of fish aroused much interest because of their wide distribution and their economic importance. During this time hundreds of papers dealing with the protozoal parasites of fish appeared. The end of the nineteenth and the beginning of the twentieth century have seen much expansion of our knowledge of the protozoa of fish and today hundreds of different species of parasites are included in some genera, families and classes of two sub-phyla of protozoa. They are a very large, comprehensive and important group of organisms which exhibit a wide range of variation in developmental and structural characters.

During the accumulation of this literature much has been learned of various parasites and their affinities. The great need is for consolidation of this knowledge and more detailed investigation. With these needs in view the present work was undertaken. As the investigation progressed, it became obvious that the blood-inhabiting flagellates would present a fruitful field for study.

For this purpose, Indian, British and European fish were collected. On examination and investigation various parasites of the classes Hastigophora, Sporozon and Ciliata were found. Hence this paper is divided into three parts. The first part deals with the life cycles of trypanosomes and trypanoplasms in culture obtained in artificial medium, the life cycle of certain flagellates in vector leeches and the detailed morphology of some organisms which had been described but briefly by previous investigators. The second part gives a description of the life history of some of the Myxosporidia which are of known species, and others unknown. Myxosporidia have been known for nearly one hundred years and consequently a large number of papers have been written by various observers. Nearly 95 per cent of Myxosporidia are found in fishes although they occur occasionally in Amphibia and Reptilia. A great majority of these parasites have been discovered in the fish of France, Germany, Italy, Switzerland, England, the United States, Brazil and Japan, although they have been found everywhere in the world where microscopical work has been carried out.

The third part consists of a description of ciliates which live on the external surface of the fish, while a few intestinal ciliates have also been seen in some fish.



Some ciliates when present in great numbers may cause the death of the fish.

#### FUTURE FIELD OF STUDY AND INVESTIGATION.

At present the group of blood-inhabiting flagellates may not be the most alluring to a prospective investigator. This is probably because much work has been done on this group, and hence the prospects of further experimental work are less promising. Nevertheless, the comparative study and profound changes of the trypanosomes of fish when the latter are kept on experimental and ordinary mixed diets are worth investigating. The writer has observed a large number of trypanosomes in the blood of the Indian fish, Clarias batrachus, which were fed only with bread for three weeks while the reverse held in the case of those which were given earthworms.

In comparison with European and American countries comparatively few publications on parasitic protozoa have appeared in India and the number of recorded hosts and parasites is not large. Intestinal flagellates have also not been found in freshwater fish. Hence there is much scope for investigation of the intestinal flagellates and for artificial cultivation if possible.

Another problem is, can fish flagellates be introduced into other cold blooded vertebrates and the converse can flagellates of cold blooded vertebrates



develop in fish when they are inoculated by vectors? Such experiments concerning the transmissibility of trypanosomes of fish to other vertebrates and vice versa remain to be investigated.

Haemogregarines occur in both red and white blood corpuscles of fish and in leeches though they are found much less frequently in freshwater fish, than in marine ones. Eels and other fish from Portugal, the river Nile and rivers of Paraguay harbour some haemogregarines but little is known of their life-history.

Coccidia from fish can be studied in detail. They have been found among others by Thomson & Robertson (1926a, 1926b). They stated that the oocysts of fish coccidia have been eaten by the human host, passed through the intestine and been seen in the faeces. Other problems are open for study involving the host-parasite relationships of members of this group.

Among the Myxosporidia there are many problems to solve, such as:

1. Artificial culture - No one has succeeded in cultivating Myxosporidia in any medium though some observers have studied certain changes which these parasites undergo when subjected to the digestive fluid of the host fish.

2. Infection through the digestive tract - Experiments can be done by introducing gill filaments of infected fish containing the myxosporidian cysts into the alimentary canal of another fish. Thelohan (1895) observed infection taking place through this system.

3. Viability of the spores - How long the spores live under various circumstances is very little known.

4. Seasonal occurrence - The great majority of Myxosporidia were discovered during the warmer months of the year. Some investigators continued observations throughout the year but the data are too meagre and inadequate to draw any conclusions.

Besides the problems mentioned above there are many others such as problems of auto-infection, effect of the histozoic Myxosporidia upon the host body, the origin of the sporoblast or pansporoblasts, modes of infection and problems of immunity.

In the Ciliata one can study the development of ciliates found on the external surface or in the intestine. Many organisms of this class have been found but little is known about the earlier developmental stages.

In brief, the field of research on the various phases of the subject in question is very wide as well as interesting and there is much to be added to our

information on protozoa of freshwater fish, which may be of great interest and value, not only to the investigators but from an economic point of view,

#### MATERIALS AND METHODS

The materials used for the investigation have been obtained from fishes collected from different sources. Seventy-three air-breathing freshwater fish were brought from Hyderabad State, India. A large number of fish were freshly caught from two lakes at St. Albans and from Windermere, Great Britain. Other fishes, British and European, were bought at local fish shops and from Trout Fisheries in Essex.

The list of the fishes examined is as follows:

P.T.O.



British fish	No.	European fish	No.	Indian fish	No.
1. Bream ( <u>Abramis</u> <u>brama</u> )... 31		1. Eel ( <u>Anguilla</u> <u>vulgaris</u> )... 70		1. Maroof ( <u>Clarias</u> <u>batrachus</u> )... 30	
2. Bullhead ( <u>Cottus</u> <u>gobio</u> )... 15		2. Mirror carp... 15		2. Singhi ( <u>Saccobranchus</u> <u>fossilis</u> )... 25	
3. Carp ( <u>Cyprinus</u> <u>carpio</u> )... 30		3.		3. Murrel ( <u>Ophicephalus</u> <u>striatus</u> )... 18	
4. Char ( <u>Salvelinus</u> <u>willoughbi</u> )... 29					
5. Loach ( <u>Cobitis</u> <u>barbatula</u> )... 6					
6. Minnow ( <u>Phoxinus</u> <u>phoxinus</u> )... 15					
7. Perch ( <u>Perca</u> <u>fluviatilis</u> )... 12					
8. (Pike ( <u>Esox</u> <u>lucius</u> ).... 10					
9. Roach ( <u>Rutilus</u> <u>rutilus</u> ).... 17					
10. Rudd ( <u>Scardinius</u> <u>erythrophthalmus</u> ) 6					
11. Tench ( <u>Tinca</u> <u>tinca</u> )... 4					
Total	175	--	85	--	73

For observation of living organisms hanging drop preparations were made. Some fresh films in normal saline with methyl blue and eosin were examined. Numerous blood and bile smears, teased nodules and scraped scale preparations were employed. Wet fixation with osmic acid vapour, Schaudinn, Flemming & Carnoy fixatives were used. Staining was carried out with Leishman, Giemsa & Heidenhain iron haematoxylin (alcoholic and watery) stains.

The extrusion of the polar filaments of the Myxosporidian spores was caused by addition of potassium hydrate.

All drawings have been made with the camera lucida to a scale in which 10  $\mu$  in the object is equivalent to 25 m.m. in the drawing (X 2500).

Under the relative sections further details of technique are given where necessary.

#### HISTORICAL ACCOUNT OF TRYPANOSOMES OF FISH.

Investigations have been carried out since before the second half of the 19th century on the blood trypanosomes of freshwater and marine fish from various parts of the world but especially in European countries.

Valentin (1841) (Berne) was the first person to discover a trypanosome in the blood of a freshwater fish, Salmo fario. A year later Remak (1842) observed flagellata in a pike (Esox lucius) and in many other freshwater fish. Gros (1845) found trypanosomes in several species of fish. Since then a large number of trypanosomes has been noted in fish in many countries and many of them have been given specific names. The list of fish in which trypanosomes have been found is a long one. They have been discovered and described by many observers among whom are Berg, Chaussat, Sedl, Nitrophonon, Danilewsky, Chalachnikov, Kruse, Lingard, Salreges, Laveran and Mesnil, Hofer, Leger, Plehn, Brumpt, Wenyon, Minchin, Robertson, Fantham and Tanabe. The descriptions given by these workers have varied greatly in completeness from a mere mention to reasonably complete descriptions.

The first satisfactory account of the occurrence of trypanosomes in fish was that of Danilewsky (1888) in the carp. Since then many of these flagellates have been described, inadequately in the great majority of cases. Petrie (1905) described his observations relating to the structure and geographical distribution of certain trypanosome of goldfish. Minchin (1909) gave a very satisfactory account in detail of the incidence of certain trypanosomes and trypanoplasma in British freshwater fish illustrated by beautiful diagrams.



Thomson in 1908 published a remarkable account, for the first time, of the development of the trypanosome of goldfish (carp) in culture obtained on NNN medium. Robertson (1911) recorded her experiments on the transmission and life cycle of certain flagellates in the intermediate host the leech, Hemiclepsis marginata. Indeed a considerable amount of work on the trypanosomes in the blood of fish has been done but little attention has been paid to the life cycle of trypanosomes in culture.

(PART I).

THE MORPHOLOGY AND LIFE CYCLE IN THE HOST  
AND IN CULTURE OF TRYPANOSOMA STRIATUM,  
A PARASITE OF AN INDIAN FRESHWATER FISH.


Introduction

This study is based on observations on trypanosomes inhabiting the blood of an Indian fresh-water fish Ophicephalus striatus and on various developmental stages in culture grown upon blood agar. The work was undertaken because of the brief account of this parasite, without nomenclature, previously given by Lingard (1891-93) and Mathis and Leger (1911). The fish in question were brought alive from India along with fifty five of two other species belonging to the family Siluridae.

Trypanosoma of Ophicephalus striatus

In all, eighteen Ophicephalus striatus, an air-breathing fresh-water fish of India have been examined. Of these five were found to harbour trypanosomes in their blood.

(a) Morphology of forms found in the host.

In fresh films the organism can be detected by its motility amongst red <sup>and</sup> white  blood corpuscles.

Living preparations.

Two forms, a small and a large are seen, the small being very actively motile while the large appears stout and sluggish.

Stained preparations.

In blood preparations stained with Giemsa, the flagellates are markedly polymorphic, namely small (fig 1), intermediate (figs 2-8) and large (figs 9-12), the latter being very broad and stumpy and by far the commonest. The smaller the body the denser and more stainable is the cytoplasm so that the stain largely obscures the details of structure.



Shape.

The body of the parasite as found in the host is a sinuous, elongated, fusiform structure. The anterior end is attenuated and pointed; the posterior end is less so and ends rather abruptly in a more or less rounded extremity.

Cytoplasm.

The cytoplasm in the smaller form, as mentioned above, is usually densely granular with a very few vacuoles but in the larger forms less granular and with many vacuoles. The type intermediate in size has characters similarly intermediate in degree. The granules in question are more or less irregular in shape and tend to be clustered together in a compact mass. No myonemes have so far been seen in the body.

Nucleus.

The nucleus generally lies in the anterior half of the body and rather near to the anterior end. It is dark red in small forms and often very difficult to differentiate from the surrounding densely stained cytoplasm. In large stout forms it is very clearly

defined with light staining. Generally the nucleus is spherical or oval (fig 10), pear-shaped (fig 11) or bean-shaped (figs 3, 5 & 9). Its chromatin granules vary in size according to the size of the organism being much larger in stout forms. In some, a distinct karyosome stained deeper red than the rest of the nucleus, is present. Granules are either scattered or arranged in rows (figs 5 & 7), in the cytoplasm which gives an impression of myonemes. This arrangement is not found in intermediate and small forms but it would, in any case, be difficult to distinguish due to the deep staining.

#### Parabasal body.

The parabasal body appears oval or rounded. It varies in size corresponding with the size of the parasite and lies a short way in front of the posterior extremity. A distinct large vacuole is present connected to the parabasal body. In fig 3 it is lightly stained.

#### Blepharoplast.

No blepharoplast so far has been observed in any specimen, as the axoneme appears to arise directly from the parabasal body.

Flagellum.

The flagellum seems to originate from the parabasal body. It is seen clearly running along the border of the undulating membrane and the distinct free flagellum is longer in parasites of small and intermediate size than in large stumpy forms.

Undulating membrane.

The undulating membrane is narrow in all forms with many small folds. It does not become wider with increase in size of the body of the parasite. In some cases the folds in the narrow undulating membrane are very long (fig. 7) but usually they are short in all the forms. The membrane is completely hyaline in structure.

Dimensions.

The dimensions of the parasite are as follows:

P.T.O.



No.	Particulars	Small forms	Intermediate forms	Large forms
1.	Posterior extremity of the body to posterior border of the nucleus..	13.0 $\mu$	15.5 $\mu$	26.0 $\mu$
2.	Posterior to anterior border of nucleus (length of the nucleus).	2.5 $\mu$	3.75 $\mu$	5.75 $\mu$
3.	Anterior border of nucleus to anterior extremity of the body. .. ..	16.5 $\mu$	19.75 $\mu$	23.0 $\mu$
4.	Breadth of the body at its widest part. .. ..	2.0 $\mu$	3.0 $\mu$	8.0 $\mu$
5.	Length of free flagellum.	8.0 $\mu$	10.25 $\mu$	11.0 $\mu$
6.	Total length of the body.	40.0 $\mu$	49.25 $\mu$	65.75 $\mu$
7.	Breadth of nucleus. ..	1.5 $\mu$	2.5 $\mu$	7.0 $\mu$
8.	Length of the parabasal body. .. ..	0.75 $\mu$	1.0 $\mu$	1.75 $\mu$

Stages of division have not been observed in living preparations nor in stained films. Organ smears revealed no trypanosomes.

#### Systematic position.

Lingard in 1891-93 discovered trypanosomes in Ophicephalus striatus along with two other species of

fresh-water fish, obtained from the river at Poona, South West India. No morphological characters, dimensions or diagrams of the parasite were given by him. He merely noted the following points.

"Two species of Trypanosoma were observed, one a large variety and the other a very small one. The former was frequently present in the circulation of Ophicephalus striatus. In every instance the most marked development of the undulating membrane was observed."

He also did not give any name to the trypanosome he found. It is probable that the parasite mentioned above was the same as that observed by the writer in the same species of fish brought from India.

In 1908 Wenyon recorded the presence of trypanosomes in the blood of Ophicephalus obscurus, a mud-feeding fish which abounds in the Nile river and especially in Lake Ambadi. He stated that the African trypanosomes measured 40  $\mu$  in length, 3-4  $\mu$  in breadth while the flagellum was about 4  $\mu$  long. The parabasal body was situated at the extreme posterior end and the nucleus was behind the middle point of the body. The Indian trypanosome differs from the African in form, dimensions, position of parabasal body, nucleus and in the length of free flagellum noted above.

Mathis and Leger in 1911 also reported the occurrence of trypanosomes in two freshwater fish namely Ophicephalus striatus and Ophicephalus maculatus in Tonkin. They gave a brief account of the parasites with dimensions but without figures.

The trypanosomes of Ophicephalus striatus they recorded were of two different varieties namely variety (A) small and variety (B) large. The form (A) had a long flagellum, (B) a short one. The protoplasmic body was vacuolated and the folds of undulating membrane numbered 5 or 6. This description is too brief to form any definite opinion upon. The trypanosomes found in the Indian fish, are polymorphic, larger in size (a small trypanosome is larger than the large trypanosome of Tonkin), and there are differences in position of nucleus and parabasal body.

As for the trypanosome of Ophicephalus maculatus, it was described as being of two types, young and old. The body was long and narrow in the young forms. The nucleus was composed of chromatinic granules, the parabasal body was subterminal and the undulating membrane, slightly folded, was narrow and connected to the body.

This trypanosome of Ophicephalus maculatus somewhat resembles the trypanosome of the Indian fish in position of parabasal body which is subterminal and



in having a narrow connected undulating membrane but differs in dimensions and other respects.

Mathis and Leger gave no specific name to the parasite they observed nor did they figure it. Taking all the foregoing facts into consideration, it is proposed to name the trypanosome of the Indian fish Trypanosoma striati with specific characters as described in this account.

(b) Cultivation of Trypanosoma striati.

Some earlier investigators have attempted to cultivate trypanosomes, found in the blood of fish, in NNN medium. Petrie (1905) removed blood containing Trypanosoma danilewskyi from the heart of a goldfish and placed it in blood agar medium. The tubes were kept at room temperature and in ten days cultural forms appeared. He noted that in shape they were tadpole like. On the 11th day a group of four individuals was seen on the 12th day one with five individuals. On the 13th day the trypanosomes were much fewer. Subcultures were made but without success although a considerable inoculum was added to each culture tube.

In 1906 Brumpt tried to cultivate the trypanosome of the eel in NNN medium but did not succeed. In the same year Lebailly, contrary to the experience of Brumpt, observed some dividing forms of trypanosomes in a film of blood between slide and coverslip sealed with paraffin wax and kept for eight or ten days. He did not use any medium at all and so was unable to make subcultures.

Thomson in 1908 succeeded in cultivating trypanosomes of goldfish in NNN medium slightly modified by Nocht and Mayer. He prepared the medium according to the formula recommended by the above-mentioned workers, and inoculated the infected blood from the heart of a goldfish with aseptic precautions and kept it in a cool place at a temperature of about 15°C. He found a growth of trypanosomes when he examined the culture seven days later. The same culture tubes were also examined at intervals of twenty one, twenty eight and forty three days and he described the various developmental forms of trypanosomes in detail along with figures. After forty three days he made a subculture and went on holiday for seven weeks. On returning he found neither the original culture nor the subculture in existence.



Tanabe (1924-25) introduced trypanosome-infected blood of a Japanese fish into NNN medium but was unable to obtain a culture. He then used Ponselle's medium and succeeded in his attempt and then remarked that trypanosomes did not proliferate in NNN agar but thrived only in Ponselle's medium.

In view of the fact that several workers have obtained cultures of trypanosomes of fish yet could not maintain them for any length of time the writer attempted to make cultures for use in elucidating certain points and to carry out certain experiments. The points considered to require elucidation or study are given here.

1. The morphology and life cycle of trypanosomes in culture.
2. Temperature in relation to culture.
3. Effect of salt solutions of different concentrations on trypanosome cultures.
4. Survival of trypanosomes from cultures in the blood of different species of fresh-water fish when injected into the heart or muscle.
5. Feeding of leeches on cultures of trypanosomes.
6. Production of cultures from isolated single trypanosomes present in cultures.
7. Effect of reagents on trypanosome cultures.

(1) Methods of cultivation

The trypanosome now being dealt with, T. striati was cultivated in blood agar. The following media were prepared according to the formulae given by the authors:-

1. NNN medium.
2. Shortt's modification of NNN.
3. Nocht and Mayer's modification of NNN.
4. Noller's medium.
5. Row's medium.

For obtaining the primary culture of trypanosomes all the above mentioned media were found unsatisfactory except Shortt's modification in which the trypanosomes of Ophicephalus striatus and Clarias batrachus and trypanoplasms of the British fish Salvelinus willoughbi the Windermere char, have been grown abundantly and are still being maintained in subculture after 16 months, at 22° and 15°C. As far as the writer knows this is the first time that these cultures have been maintained for long periods of time.

After having prepared the medium recommended by Shortt the infected Ophicephalus striatus was killed by a blow on the head and opened up with full aseptic precautions by painting the whole body with tincture of iodine solution. The blood from the heart was then taken

with a sterile pipette and inoculated into five medium tubes and a small piece of spleen was also put in a sixth tube. All tubes were kept in the incubator at a temperature between  $20^{\circ}$  and  $22^{\circ}\text{C}$ . for a week. When examined after a week the tube containing spleen tissue was found contaminated with bacteria. In three tubes the original trypanosome forms were observed, and the remaining two were negative. The five tubes were returned to the incubator for another fortnight. On the twenty first day three tubes were positive with a rich growth of trypanosomes and two negative though they were not contaminated. These two tubes were again examined after another week with negative results. Subcultures from the positive tubes were made into fresh medium.

In order to study the life cycle, solid medium (plates) of Shortt's, Noller's and that used by Thomson were prepared. Plates were inoculated by putting a drop of the culture with a pipette upon the surface of the solid medium and spreading it with a sterile L shaped glass rod. This petri dish containing the medium was then covered by another petri dish larger in size and then was turned upside down. In this way the medium dish was upside down with the empty large dish below it.

Saturated mercuric chloride solution was poured into the empty dish to prevent subsequent contamination of the plates. Several coverslip impressions were taken every day up to nine days and then on the 11th and 19th day.

(11) Micro-isolation technique.

After the culture of trypanosomes was established in Shortt's modification of NNN an attempt was made to isolate a single parasite from each of the cultures in question and to introduce it into fresh medium tubes with the object of producing cultures of clones. The following two micro-isolation techniques were applied:-

1. Dilution method with citrate saline.

For the dilution method, four sterile clean glass slides were taken and on each slide, well protected from contamination, four separate drops of condensation fluid of the medium were put. Thus sixteen drops were put on four slides. Afterwards a loopful of culture was mixed in the first drop. From this diluted drop a loopful of fluid was diluted in the second drop and then from second to third and so on in rotation up to the sixteenth drop. All the drops were then examined serially for the organism. Some of these drops contained six to eight flagellates



while others had one to three, but the last two were found with one organism only. If the last drop contained more than one parasite, it was again diluted with another one or two fresh drops of condensation water until one organism only was found. The whole fluid of these last drops was immediately inoculated with a sterile pipette into medium tubes.

This procedure was carried out six times and the tubes were then kept in the incubator at 22°C. temperature. After three days one loopful of fluid from each tube was examined with negative results. The tubes were then again kept in the incubator for a further seven days. When examined on the eleventh day one tube was found with bacterial contamination, three gave negative results and two were positive with moderate growth.

This experiment indicated that the one isolated trypanosome succeeded in multiplying in the medium. Unfortunately the writer did not make any note of the individual form introduced into the medium whether young, long, or stumpy, so the experiment had to be repeated again to make sure which form was capable of growing and multiplying. These isolated parasites when inoculated into the medium proved to be crithidial forms.

## 2. Capillary pipette method.

The culture was diluted in the same way as mentioned in the first method and the last drop was drawn into a very fine sterile slender thread-like pipette and immediately checked under the microscope for the presence of a trypanosome before being dropped into culture medium (low power with x17 eyepiece). This method was not found satisfactory.

## Fixation and staining of cultural forms:

For wet preparations Schaudinn's fixative and Flemming fixative were used and the slides stained with Heidenhain's iron haematoxylin with different degrees of differentiation. This stain was very satisfactory in showing the structure of the nucleus.

Dried films were fixed either in absolute alcohol or methanol for five to ten minutes and were stained with Giemsa stain in various proportions, that is to 1 c.c. of buffered distilled water 1, 2 or 3 drops of stain were added and staining carried out for half an hour to two hours. Some films were stained with phosphotungstic haematoxylin stain but this was found unsuitable for the writer's purposes because the flagella were indifferently stained.

Hanging drop preparations were also made to observe the division of the trypanosomes in the medium fluid. This observation will be described subsequently.

Observations made:

Movement: The movement of cultural forms has been observed in hanging drop preparations. Small free actively motile forms have been seen. Some pass rapidly across the field of a low power objective. They change their direction only when they are checked by any particle or come in contact with another form. Some move round and round with the posterior end attached to the surface of the slide. A few individuals wriggle, roll up and twist their bodies in a screw like manner. Long and attenuated flagellates move in a straight line as though towed. When dividing the mother forms always drag their daughter individuals behind them either in a straight line or on an irregular zigzag course. Large and stumpy forms with several flagella are very sluggish but have very active flagellar movement.

(c) General account of cultural forms and life cycle:

Shape of the Trypanosomes in culture:

Trypanosomes in culture are polymorphic. In the solid medium the morphology varies greatly. In fluid, generally they are less broad, somewhat spear-headed and

tadpole like for the first four days but from the fifth day onwards they become broader and swollen posteriorly. Many dividing forms are irregular in shape, the mother individuals with broad, bulky and obtuse posterior end while the daughter individuals are bud-like. Some are dumb-bell shaped. A few very slender attenuated individuals have been seen in the earlier days of subculture. In solid medium many different shapes can be seen. Earlier, in one or two days, they appear fusiform with both ends tapering but from three days onwards, many of them exhibit typical and marked changes in their shapes. Some are sub-spherical and globular (fig. 17) with the anterior end somewhat pointed and the posterior end rounded. The nuclei are situated in the centre (figs. 17 & 20). Certain forms are oval or pear-shaped (figs. 18 & 20). A few are lancet-like (fig. 19) and in some, a hood-shaped posterior portion of the body simulates a cobra (fig. 14). In these the nucleus is in the broad posterior end (figs. 14 & 69). Other forms are rectangular, club-shaped or dumb-bell like (fig. 20 & 21).

#### The cytoplasm:

The cytoplasm within the periplast is finely granular. In Giemsa preparations the cytoplasm is very



conspicuous with granules which are situated at the posterior end of the body. The anterior end contains less numerous granules with a few scattered vacuoles. In some, equally distributed granules have been seen at both ends. They are sometimes clustered together and stain a deep red with Giemsa. When observed in fresh preparations, the granules appear to move to and fro due to the movement of the organism. In iron haematoxylin preparations, on the contrary, the granules are not seen and the cytoplasm appears to be hyaline. Vacuoles of various sizes are seen throughout the whole body but most markedly in the posterior portion. Large numbers of vacuoles have been observed in the preparations made from solid medium where the bodies of the organisms are more expanded; this is even more marked in degenerating forms.

Thomson (1908) has described the granular condition of the cytoplasm, when seen in Giemsa stained preparations twenty eight days after culture and considers it might be due to some influence of the medium or, most probably, to the result of some accidental change that had taken place in it, and that it might possibly be analogous to the granular condition of most cells. The writer has also seen the coarse granules in the parasite

in the medium used by Thomson (not in the fluid form, as he examined them in but on solid blood agar) and stained with Giemsa, while preparations from Shortt's and Noller's media showed a lesser number of granules. Therefore it can be said that the granular condition might be due to some influence of the medium. In every preparation from the above mentioned media with iron haematoxylin stain, the coarse granules were not seen. Besides this, conspicuous vacuoles are more often seen in the cytoplasm in Thomson's medium than in the other two.

In a few young forms a prominent cleft like streak has been observed in the posterior portion of the body (fig. 80). This streak passes obliquely backwards towards the parabasal body. Similar streaks may be seen in other parts of the body.

One peculiar appearance often seen in fresh films is a conspicuous line of demarcation in the cytoplasm just before the parasite is about to divide. When the parabasal body and the new small flagellum have been formed, a line appears in the cytoplasm and slowly the cytoplasm begins to divide along the same line.

This line has been seen in films stained with iron haematoxylin (fig. 61) but in some Giemsa

preparations there is a distinct differentiation between mother and daughter individuals owing to dark and light staining respectively, the daughter form being the darker so that the actual line may be marked.

### The organelles.

The nucleus: The nucleus generally is oval (fig. 20) or circular (figs. 17, 19, 20 & 71), in shape in small and large stumpy forms but compressed and elongated in long and attenuated types (figs. 99 & 101).

When examined in fresh preparations the nucleus appears somewhat darker than the cytoplasm. On close observation a darker structure, the karyosome, is seen towards the centre of the nucleus.

After an iron haematoxylin stain with a moderate degree of differentiation the nucleus appears as an oval or round, black area surrounded by an achromatic margin (figs. 28 & 29). With further differentiation the black area becomes a small central dark spot, the karyosome (figs. 13 & 14) with a lighter staining peripheral portion<sup>which</sup> is rather darker than the cytoplasm but on further differentiation it becomes a clear halo-like zone surrounding the dark karyosome while its periphery remains as a dark line.



Sometimes one or more round bodies, probably grains of chromatin, are seen in the space between the nuclear membrane and the karyosome (figs. 89 & 91).

In a few forms radiating fibres connecting the karyosome with the nuclear membrane have been seen in preliminary degrees of differentiation of the iron haematoxylin stain (figs. 78 & 79) which Robertson (1937) has seen in the large Trypanosoma rajae in the blood of marine fish and in the same trypanosome in the leech (Pontobdella muricata).

After much differentiation the karyosome appears to be of two types, I and II. In type I, it appears as a conspicuous round body nearly one half or one third the diameter of the nucleus (figs. 13 & 14). In type II, it appears as two delicate masses connected with a rod-like structure which is possibly a centrodesmose.

Variations seen are as follows:

1. The karyosomic mass is connected to a rod giving<sup>a</sup> drum stick-like appearance (figs. 60 & 63).

2. The karyosomic mass is divided and the masses lie at either end of a centrodesmose, thus resembling a dumb-bell (fig. 83).

3. Various transitional stages with a short centrodesmose (figs. 85 & 86).

4. Two equal masses joined together to form a bilobed karyosome (fig. 87).

All these above-mentioned forms have been seen in stumpy forms of the trypanosome growing on solid medium. In the forms in fluid medium these changes are very/rare, or at least difficult to see.

In nuclear division, as generally seen in wet fixed preparations, there appears at first a rod or ribbon shaped structure associated with the karyosome (figs. 26, 27, 65 & 69). The nuclear membrane and karyosome then elongate and become con<sup>s</sup>tricted at the centre. At this stage the nucleus looks dumb-bell shaped (figs. 22 & 62). Further elongation takes place and the halves of the divided karyosome occupy the poles and are linked together by a fine dark thread or line (figs. 22, 56 & 57). Finally the link disappears and two daughter nuclei are formed (figs. 53 & 68). The newly formed nuclei become round and globular and similar to the original one.

Kinetoplast: The kinetoplast consists of:

- a. The parabasal body.
- b. The blepharoplast.
- c. The flagellar vacuole.
- d. The rhizoplast.



Parabasal body: The parabasal body varies in shape and size in different forms. The shape may be round, globular, (figs. 14, 81, 82 & 90), oval (fig. 76), triangular (fig. 97) or rod-like (figs. 58, 66 & 67). It lies at the posterior end of the mature trypanosomes (figs. 96, 97 & 98). In the immature or crithidial forms it is situated close to the nucleus, either in front of it or behind or on either side (figs. 33, 65, 75 & 83). In size it is about one third the diameter of the nucleus and in some it is of the same size as the karyosome (figs. 81 & 82).

Blepharoplast: A small dot like mass is situated in front of the parabasal body and from it the flagellum arises (figs. 58, 66, 67 & 70). It is very difficult to see this minute structure in any form in culture but after a prolonged search, the writer was able to see it in a few forms.

Flagellar vacuole: The flagellar vacuole is clearly seen in stained films. It is a pale area in an iron haematoxylin preparation and of pink colour in Giemsa. It lies in front of or closely connected to the parabasal body, is generally well defined in crithidia and dividing forms and perhaps absent in mature trypanosomes.

Rhizoplast: The rhizoplast or basal portion of the flagellum usually appears as if it originated from the parabasal body. Before the new flagellum arises, there appears a vacuole like appearance in the rhizoplastic area (figs. 55 & 66) preliminary to the formation of a new flagellum. At first a minute darkly-stained dot is seen in the position to be occupied by the root of the new flagellum (figs. 66 & 70). Then the changes in the parabasal body and the original rhizoplast consist of separation of the two parts of the parabasal body and the elongation of the newly formed rhizoplast to form a complete flagellum (fig. 67). Gradually the new and original parabasal bodies separate more widely from each other (figs. 26, 30 & 43). The newly formed rhizoplast, after reaching the surface of the body increases in length further to form the new flagellum. In some cases the new free flagellum has been seen before the division of the parabasal (fig. 39).

Flagellum: The flagellum, a long filamentous protoplasmic extension, appears uniformly stained. In dry films stained with Giemsa it is thicker than the flagellum stained in iron haematoxylin. The thickness in the former preparation is due to the

staining of both the axial filament and its enveloping contractile protoplasm. It may be about one sixteenth or more of the body breadth in Giemsa stain while it is half of the former in iron haematoxylin. No distinction between axial filament and protoplasmic envelope so far has been seen in any form of trypanosome culture.

The flagellum in wet-fixed films appears to commence from the parabasal body (figs. 54 & 90) but in certain cases, in preparations from solid blood agar there seems a short gap between the parabasal body and the origin of the flagellum (fig. 58).

Usually the flagellum at its base passes through the flagellar vacuole. Sometimes it appears to pass on either side of the vacuole.

In dividing forms two flagella are seen of which the newly formed is the smaller. They are usually unequal even on complete division but in some cases they appear to be equal in length.

Division: The occurrence of division in all stages in culture is obvious but it is difficult to present in correct sequence the details of the gradual changes in the parasite. Multiplication occurs in all types by longitudinal fission which commences with division of the blepharoplast and as soon as it has separated into



two, a new flagellum grows out from the daughter blepharoplast. The writer was only able to see one blepharoplast which appeared to be in division.

To see the divisional forms cultures made in fluid and on solid blood agar (plates) were used and smears were stained in Giemsa and iron haematoxylin. The latter medium was found most suitable and satisfactory. In these preparations nearly all the changes of development were well displayed. The parasites are deformed in dried Giemsa-stained films as they are flattened on the surface of the slide and appear larger than in the wet-fixed films. Besides this, details of nuclear structure, are obscure although the parabasal body as a solid or bilobed mass in such films can be studied.

The same experience has been gained with Giemsa preparations of coverslip impressions from plate cultures. To obtain the best results the writer used Flemming's fixative in which the smear when made is immediately dipped face downwards for five minutes. The slide is then washed in tap water and stained with Giemsa.

In division, the primary changes occur in the blepharoplast and parabasal body then in the nucleus. Finally the cytoplasm divides by longitudinal fission commencing anteriorly.



Changes in the parabasal body:

In the individual free form the parabasal body is a single mass, but at the time of division the body elongates and assumes a rod or ribbon like appearance. It then constricts in the middle and becomes bilobed, (figs. 16 & 59). The first flagellum at this time remains either between the two lobes or goes to one of the lobes (fig. 16). Later a minute structure shoots up from the base of the original flagellum and moves to the newly formed parabasal body (derived from division of the original bilobed parabasal body). This represents the axoneme which finally grows, extends and passes through the cytoplasm either along with and parallel to the original one or runs in any other direction in the cytoplasm and becomes a free flagellum. No connecting cord between the two new parabasal bodies has been seen, but presumably must be present at some stage. By this time the two parabasal bodies have already separated and as development proceeds the gap between them and the flagella becomes wider.

The newly formed flagellum increases in length in all developmental stages of the parasite until it reaches the size of the original flagellum before the separation of the two individuals. (figs, 23, 24 & 30).

It has been stated that the new flagellum is formed by splitting of the original one but the writer has not seen any double thread-like structure at the base of the flagellum. It is possible that the very primary root of the flagellum i.e. the blepharoplast itself divides and the newly separated part gives rise to a fresh flagellum.

#### Changes in the nucleus:

The division of the nucleus occurs after that of the parabasal. As has already been described, the nucleus usually elongates laterally and widens out making a rod or ribbon shaped structure (figs. 31 & 32). This is associated with an elongation of the karyosome. The karyosome later concentrates and becomes constricted at its centre, the two daughter centrioles being connected by a fine distinct fibre which is the centrodesmose. The nuclear membrane then becomes constricted and two nuclei similar to the original one are produced.

#### Division of the cytoplasm.

After the division of the two main essential structures, parabasal body and nucleus, the splitting of the cytoplasm begins from the flagellar end of the

parasite. Usually at this time a clear linear <sup>line</sup> sulcus in the cytoplasm gradually splits along this line and finally the two dividing flagellates separate from each other and become free individuals.

Sequence of events in division:

In the previous pages it has been stated how the division of the parabasal body, the flagellum, the nucleus and the cytoplasm takes place gradually one after the other. This simple method of fission is not constant but there appear on occasions further subdivisions of internal structures before the splitting of the body is completed. Hence the phenomenon becomes a complex one and the correct sequence of the events in division is not always obvious. This is because of occasional independent division of the organelles, which results in multiple fission of the parasite. For example, the parabasal body after primary division undergoes further subdivisions before the nucleus and the cytoplasm have divided and separated.

Details of division of various structures which have been studied are recorded in tabular form in an attempt to correlate the timing of the various stages of division in the organelles to the process as a whole.

Stage	Parabasal body.	Flagellum	Nucleus	Cytoplasmic body.
1.	Single	Single	Single, no change.	Fusiform or tadpole like.
2.	Elongated	do	do	do
3.	Bilobed	Original on either of the two.	do	do
4.	Divided.	do	do	Posterior body swollen.
5.	Separated	New flagellum shoots free from lobe.	do	do
6.	do	Further growth	Rod-shaped	do
7.	do	do	Constriction and dumb-bell shaped.	do
8.	do	do	Further constriction.	do
9.	Widely separated	do	Connected with centrodesmose.	Irregular on fusiform with posterior body swollen.
10.	do	2/3rds original in length.	Separated.	Body divides.
11.	do	do	Widely separated.	do
12.	Passed towards posterior end or near the nucleus.	Fully developed.	do	Further division.
13.	do	do	do	Complete division with two daughter individuals.



(d) Types of parasite in culture:

Various types of cultural flagellates have been noted in fluid as well as on solid media of Shortt, Holler and Thomson. The parasites which thrive in fluid are of two or three types while those on solid media are of many forms. The types are as follows:

1. Fusiform.
2. Long attenuated.
3. Tadpole like.
4. Small mature.
5. Globular and subglobular.
6. Stumpy.
7. Long multiplicative.
8. Large with multiple fission.
9. Degenerative.

In the above mentioned types, except Nos. 2, 4 & 9 nearly all show the crithidial phase but a very few are leptomonas forms.

1. Fusiform type: These forms may show all developmental stages of continuous growth. The cytoplasm stains deep purple in Giemsa and appears reticular in iron haematoxylin. The nucleus is generally oval or round, lies in the posterior half of the body. In crithidial forms the parabasal is situated anterior to and very near the nucleus. The

flagellum runs from behind forwards through the cytoplasm but not along the side of an undulating membrane. The undulating membrane in most cases is not present but in a few forms one or two folds are seen. The dimensions of the parasites are: Body length 10.5 to 12.8  $\mu$ ; body breadth 2.0 to 2.8  $\mu$ ; nucleus 1.4 to 1.8  $\mu$ ; and free flagellum 5.0 to 10.0  $\mu$  (figs. 33, 65, 90 & 92).

2. Long attenuated type: The body appears very attenuated and long in which the cytoplasm is similar to the type No.1. The nucleus stains deeply in Giemsa. It is elongated and oval. The parabasal body is seen with difficulty due to deep staining of the cytoplasm. The flagellum is comparatively long. The undulating membrane is inapparent. The length is 25.0 to 40.0  $\mu$ ; breadth 1.0 to 1.5  $\mu$ ; nucleus 2.5 to 3.0  $\mu$ ; free flagellum 10.0 to 15.0  $\mu$  (figs. 99, 100 & 101).

3. Tadpole-like type: In this type the posterior two thirds of the body is swollen with the end round and blunt, while the anterior third is unaltered. The nucleus is loose in structure as stained in Giemsa, but round to oval in iron haematoxylin. At this stage, apparently division can take place. It is 10.2 to 14.2  $\mu$  long and 12.5 to 14.2  $\mu$  broad. The nucleus is 1.2 to 1.5  $\mu$  and free flagellum 4.0 to 6.0  $\mu$  (figs. 80, 87 & 89).

4. Small mature type: A very few cultural forms become mature trypanosomes with the parabasal body at the posterior end and the nucleus either in the centre or anterior half of the body. One or two folds of undulating membrane are present. The measurements of this parasite are as follows: Body length 10.5 to 18.5  $\mu$ ; body breadth 1.0 to 2.0  $\mu$ ; nucleus 0.8 to 2.5  $\mu$ ; free flagellum 5.0 to 12.0  $\mu$  (figs. 96, 97, 98 & 102).

5. Globular and sub-globular: The body in this type is almost rounded and varies in size. The cytoplasm is granular in Giemsa and reticular in iron haematoxylin. The nucleus is large relatively to the size of the body. The karyosome is round as seen after much extraction of the haematoxylin stain. The parabasal body is near the nucleus but generally situated close to the sides of the body. It is rod-like in this type. The flagellum is comparatively long and the undulating membrane makes one or two folds. The dimensions of the flagellates are: body length 4.5 to 15.0  $\mu$ ; body breadth 4.0 to 8.5  $\mu$ ; nucleus 2.0 to 3.5  $\mu$  and free flagellum 4.0 to 10.0  $\mu$  (figs. 16, 17 & 20).

6. Stumpy type: These are large, broad, subglobular, pear-shaped and oval forms with both ends pointed. The



cytoplasm contains small and large vacuoles and granules. The nucleus and parabasal body are very close to each other. The flagellum is fairly long. One or two folds of undulating membrane are present. The length is 10.0 to 14.0  $\mu$ ; breadth 4.0 to 6.0  $\mu$ ; nucleus 2.0 to 2.5  $\mu$  and flagellum 4.0 to 8.0  $\mu$ . Division forms are common in this type (figs. 20, 66 & 70).

7. Long multiplicative type: These elongated forms are less attenuated than the forms No.2 mentioned above. The nucleus is oval shaped. The flagellum is half the length of the body. They are 12.0 to 14.5  $\mu$  in length; 2.0 to 3.8  $\mu$  in breadth; nucleus 1.5 to 2.0  $\mu$  and free flagellum 4.0 to 10.4  $\mu$  (figs. 19, 33 & 34).

8. Large type with multiple fission: These forms are very broad and large in size and contain a varying number of nuclei and parabasal bodies with flagella. Some are oval or round, others irregular in shape. Body length 16.5 to 27.5  $\mu$ ; body breadth 8.0 to 16.0  $\mu$ ; nucleus 2.0 to 3.5  $\mu$  and free flagellum 4.0 to 5.5  $\mu$  (figs. 71 & 72).

9. Degenerate forms: These are large fusiform or irregular in shape with many small and large vacuoles.



Nuclei and parabasal bodies are in a degenerated condition and flagella average in length (fig. 104).

#### EFFECT OF TEMPERATURE IN RELATION TO CULTURE

Cultivation of trypanosomes is difficult because of two reasons, (1) the artificial medium which is a sudden environmental change to the flagellates and (2) the temperature. The trypanosomes can get used to the medium and start multiplying provided the temperature be suitable. Therefore it is important to know the optimum temperature at which the growth of trypanosomes occurs.

In cold countries fish trypanosomes can be cultivated at a temperature of 15°C., as observed by Thomson. The writer also obtained cultures of trypanoplasms of the Char from Lake Windermere at 15°C. temperature. On the contrary, the trypanosomes of tropical fish do not proliferate and thrive at this temperature. They require a higher temperature. The writer found positive cultures of trypanosomes of Indian fish in Shortt's modification of NNN medium on incubating the tubes at 22°C. temperature.

After having cultured the tropical trypanosomes it was necessary to determine at what minimum and maximum temperatures the culture could exist and grow rich. For

this purpose several tubes were subcultured and kept in the refrigerator at 4°C., at room temperature between 14 to 18°C. and in the incubator from 24° to 34°C. increasing two degrees at a time. The extreme ranges of temperature between which the growth was obtained were between 12° and 32°C. The growth was found to be rich throughout the range.

The culture kept in the refrigerator was found with very poor growth after three days. Many flagellates were dead. Those living, were less actively motile. The shape of the body appeared comma like. The anterior portion of the body became very narrow while the posterior one was swollen. The nucleus, in place of being central, was situated in the middle of the posterior swollen portion. The parabasal body was either posterior to the nucleus or on one side. A very few crithidial forms were seen. Some were pointed at their posterior ends. Hardly any dividing forms were seen in fresh preparations. Almost all parasites were dead when examined seven days later.

Tubes which were kept at room temperature 12° to 18°C. showed rich culture after three days and seven days. There were many crithidial and dividing forms. A few leptomonas forms were seen in stained preparations.

At the temperature of 20°C. the growth was found very rich after 3, 7 and 10 days. The forms were elongated with both ends tapering, but all seemed crithidial forms. Very rich culture was seen at 24°C., 26°C., 28°C. and 32°C., after 3, 7 and 10 days. At the higher temperatures it seems that multiplication and division takes place earlier whereas the growth was decreased at 34°C. with slight changes in shape in the organisms. Culture was found negative after 24 hours at 37°C. and all the flagellates were found dead.

The following figures are based on observations of the growth of flagellates at different degrees of temperature.

P.T.O.



EFFECT OF TEMPERATURE IN RELATION TO CULTURE OF TRYPANOSOMA STRIATI

The following symbols indicate rich and poor growth and absence of flagellates forms. The figures in brackets show the day of culture.

- negative: + scanty: ++ a fair number: +++ rich growth:  
++++ very rich growth.

Number of sub culture.	D E G R E E S						C E N T I G R A D E						
	.0°C.	12°	14-18°	20°	22°	24°	26°	28°	30°	32°	34°	34°	37°
45	(3)+ (7)-		(3)-										
48	(3)++ (7)-	(3)+ (7)-											
46		(3)++++ (7)+++ (10)+++		(3)++++ (7)++++									
49		(3)++++ (7)+++	(3)++++ (7)+++										
47			(3)++++ (7)++++ (10)++++	(3)++++ (7)++++									
50			(3)++++			(3)++++ (7)++++ (10)++++							
58				(3)++++ (7)++++ (10)++++			(3)++++ (7)+++						
62						(3)++++ (7)++++ (10)++++	(3)++++ (7)+++						
68							(3)++++ (7)++++ (10)++++						
70							(3)++++ (7)++++						
72								(3)++++ (7)++++					
73									(3)++++ (7)++++				
74										(3)++++ (7)++++ (10)+++			
76											(7)++ (7)+ (1)+		
80										(7)+	(2)++ (4)+	(1)- (2)-	



A STUDY OF THE LIFE CYCLE OF BLOOD AND  
CULTURAL FORMS OF THE TRYPANOSOME OF  
THE MIRROR CARP.

In this description are embodied results of some of the observations on the trypanosome found in the blood of the Mirror carp and on the culture obtained for the first time in the medium mentioned before. This was undertaken for the sake of comparison of the life cycle of this trypanosome with the development of the trypanosome of Ophicephalus striatus cultivated in artificial medium.

Material.

The fish, Mirror carp, a variety of the species Cyprinus carpio were obtained from Stambridge Trout Fisheries, Essex, which have been importing them from Germany. In the study of the parasite derived from the fishes' blood, the cultural method and blood films were employed.

(a) Description of trypanosome in the blood.

The trypanosomes in the blood are scanty and only a few specimens have been detected in stained films, but one infection was so heavy that an average of four

trypanosomes were present in each field when freshly drawn blood was observed.

In life it is a quick-wriggling organism, with the undulating membrane vibrating vigorously amongst the red blood corpuscles. It sometimes swirls round with but slight progression.

The body usually has a long, narrow crooked curve. It is pointed at both ends, the anterior end being drawn out in the form of a tail (figs. 105, 112 to 114), but in some cases the posterior end may broaden in the neighbourhood of the parabasal body. The cytoplasm is finely granular, stains lightly and contains large and deeply staining volutin granules varying in number and size. They are more frequently seen in the anterior region of the body. In a few, the granules are arranged in rows which give an impression of myonemes (fig. 106).

The nucleus is large, circular (fig. 15) or ovoid (figs. 109 and 112) to oval (fig. 105 to 107) in appearance and is definitely anterior to the central point of the body, in some being about the junction of the anterior third and posterior two thirds of the body (figs. 105, 106, 111 and 114). In stained films it is generally homogeneous and with a nuclear membrane, but an indication of a karyosome has been seen occasionally (figs. 107, 113 and 114).

The parabasal body is a well developed oval, (figs. 106, 107 and 112) spherical (fig. 110) or bar-like (figs. 105, 111, 114 and 115) mass situated at some distance from the pointed posterior extremity. In fig. 113 a projection of a portion of the parabasal body from one side appears like a short horn. There is no apparent blepharoplast present at the front of the parabasal body.

The free flagellum is long and well developed. In one specimen (fig. 108) a small swollen body is seen on the anterior extreme end of the free flagellum. The undulating membrane is conspicuous with several deep folds and is bordered by the strong flagellum.

The measurements of the trypanosome are as follows:

From posterior end of body to parabasal body.	..	..	..	..	1.5 $\mu$ to 3.0 $\mu$
From parabasal body to posterior margin of nucleus	..	..	..	..	13.0 $\mu$ to 17.0 $\mu$
Length of nucleus	..	..	..	..	2.0 $\mu$ to 3.5 $\mu$
Breadth of nucleus	..	..	..	..	1.5 $\mu$ to 2.0 $\mu$
From anterior margin of nucleus to anterior end of the body	..	..	..	..	5.0 $\mu$ to 14.0 $\mu$
Length of free flagellum	..	..	..	..	8.0 $\mu$ to 17.5 $\mu$
Breadth of body at level of nucleus.	..	..	..	..	2.5 $\mu$ to 3.0 $\mu$

Length of the body. . . . . 21.5  $\mu$  to 37.5  $\mu$   
 Total length (from tip of  
 flagellum to posterior end of body) . . . 29.5  $\mu$  to 55.0  $\mu$

Regarding its affinities, this trypanosome of the Mirror carp recalls somewhat one which Danilewsky (1885-86) first discovered in the blood of the carp Cyprinus carpio, an European fish. He gave a brief account of it without dimensions and figures. He stated there were two forms, a "simple" which was extraordinarily active, showing a wave-like motion, with small and elongated body consisting of hyaline protoplasm. The nucleus was not distinct. A line of undulating membrane was present and no differentiation between the membrane and the body could be found.

The second variety was a 'spindly' form with relatively narrow undulating membrane arranged in spiral fashion from one end to the other. A round nucleus was situated in the centre of the body surrounded by a halo. It was named Trypanosoma danilewskyi.

Laveran and Mesnil (1904) found T. danilewskyi in one carp out of four. They noted that the undulating membrane was large. The elongated nucleus was situated near the middle of the body, generally nearer the anterior than the posterior extremity. The large parabasal body was very close to the posterior end.



The protoplasm contained chromatin granules of variable number and size.

Thomson (1908) while obtaining a trypanosome culture from the goldfish species of carp gave more details about T. danilewskyi with all the dimensions and with figures.

These three descriptions throw some light on the resemblance of the flagellate of the Mirror carp in its general characters to the above mentioned trypanosome. There is for instance, the dimorphism, the position of the parabasal body, the volutin granules in the cytoplasm and the wave-like large undulating membrane with no marked differentiation between it and the body. But a close comparison of the two parasites shows a difference. The anterior end of the Mirror carp trypanosome is drawn out in the form of a tail, the posterior end often broadened in the neighbourhood of the parabasal body, the nucleus circular or ovoid to oval with the karyosome in the centre of it. Besides these points they differ in their dimensions. These features separate the trypanosome of the Mirror carp from T. danilewskyi. It is therefore designated some other name Trypanosoma winchii with characters as summarized here.

(b) Culture of trypanosome of the Mirror carp.

Hitherto, the trypanosome of the Mirror carp has not been cultivated. The writer has succeeded in cultivating the parasite from the blood in the same medium as that used for Indian fish trypanosomes.

The Mirror carp showed a mixed infection of trypanosome and trypanoplasm so it was considered to be worthwhile to cultivate both the parasites for comparison with the culture of the trypanosome of the Indian fish and the trypanoplasm of the char (British fish) already obtained.

Ten medium tubes were inoculated with infected blood with full aseptic precautions and were then put in the incubator at a temperature between 14 and 16°C. The tubes retained the normal blood dwelling forms gradually decreasing in numbers, when examined successively one, two and three weeks after the initiation of the culture. At the end of the <sup>u</sup>fourth week two tubes were found positive with cultural forms and the rest negative but without any bacterial contamination. Curiously enough the culture was found to be purely one of trypanosomes and not mixed with trypanoplasms, so it is only possible to make comparison of the European and Indian trypanosomes.

Experiments show that the multiplication phase in the vector, namely the leech, is of short duration

and is characterised by the marked polymorphism of the organisms, which measure at least  $11.0\mu$  in length and have the parabasal body near the nucleus. On the other hand, division in the medium does not take place until the lapse of one to four weeks. On the assumption that the temperature factor might account for this, a few tubes were put at  $10^{\circ}$  and  $12^{\circ}\text{C}$ . temperature, some at  $22^{\circ}\text{C}$ . and the rest at  $15^{\circ}$  and  $16^{\circ}\text{C}$ . The former two gave negative results when examined a week later, but the latter ones were found positive with parasites resembling the original normal blood-dwelling flagellates.

(c) General account of the morphology of cultural forms:

The general morphology of the flagellate stage in culture is that of a crithidia, with the parabasal body anterior to or just posterior to the nucleus and bearing a short free flagellum. There are however some other forms met with.

Shape

In the subcultures of from one day to ten days old different shapes of crithidia can be seen. When living in fluid medium there is a tendency for the organism



to assume the broad, spear-headed and tadpole like forms (figs. 116 to 118) which gradually elongate. Many are sub-globular and spherical (figs. 120, 123 to 125). Some are smaller and more slender than the blood forms measuring  $8.5 \mu$  to  $9.5 \mu$  in length whilst a few are broader (fig. 135). Stumpy trypanosomes have been observed (figs. 132 and 134). In these forms the nucleus is situated in the middle of the body with comparatively large karyosome and well developed undulating membrane. The parabasal body is rod-like in shape.

A certain number of crithidial forms (fig. 158) may be termed trypanomorphic, since in form of the body and in size they are similar to the small trypanosomes whereas their crithidial nature is manifested in the antero-nuclear arrangement of the parabasal body. They are  $16.0 \mu$  in length. Many are seen to be multiplying by equal (figs. 150 and 152) or unequal division (figs. 144, 147 and 148). Small crithidial types are also found (figs. 119, 136 and 155). The crithidia on solid medium vary much in shape, mutual relationship of the nucleus and parabasal body, in the position of the nucleus in the body and in the development of the undulating membrane. Their dimensions vary.

The cytoplasm:

The cytoplasm is finely granular and stains light blue with Giemsa stain. Numerous volutin granules are found especially in the anterior portion of the body. The iron haematoxylin preparations are finely reticular or vacuolar in structure but without any trace of volutin granules. Vacuoles are frequently seen in the posterior portion of the body (figs. 129, 135 and 137). A peculiar line often shown in the cytoplasm passes more or less directly from the neighbourhood of the parabasal body to the neighbourhood of the nucleus apparently merging with the flagellum.

The nucleus:

The nucleus is large, circular or oval in shape with a moderate degree of differentiation of the iron haematoxylin stain and appears like a black massive area (figs. 116, 117, 126, 132 and 143). It becomes resolved into showing a central portion, a karyosome, on further extraction of the stain. The space between the karyosome and the nuclear membrane appears as a clear surrounding ring (fig. 128).

The writer prepared several slides by extracting the iron haematoxylin stain to an excessive degree from

the parasites in the hope that there might be seen a centrosome but no single dot like centrosome could be seen (fig. 139). Occasionally there appear two rod like masses inside the nuclear membrane (figs. 137 and 138) which may represent the division of the karyosome without any connecting line representing a centrodesmose. The nucleus generally shows no change in its appearance until the formation of a new flagellum and separation of the two daughter parabasals has become well advanced. The nucleus then is seen as a broad ribbon within which is the karyosome also drawn out transversely (fig. 147). At a more advanced stage the band is dumb-bell shaped each head with a karyosome (figs. 149, 150 and 152) and the heads linked together by a connecting cord. After separation the newly formed nuclei assume a globular shape. The broad and stumpy forms either have circular (figs. 126 and 132) or elongated irregularly shaped nuclei (fig. 125).

#### Parabasal body:

The parabasal body varies in appearance. It is usually a round, globular, or dot like mass (figs. 118, 121 and 155) but is sometimes rod-shaped (fig. 125 and 142) or may appear triangular or heart shaped (fig. 126)



surrounded by a halo or vacuole (figs. 121, 125, 135 and 156). In division the parabasal is seen as a rod (fig. 125) or more commonly as two adjacent bodies (figs. 142 and 143) or two bodies connected by a cord (fig. 117).

#### Flagellar vacuole:

A distinct flagellar vacuole is to be seen round the parabasal body (fig. 157). This vacuole is not visible in the living condition. No blepharoplast has been seen in any specimen stained by Giemsa and iron haematoxylin stains.

#### The flagellum:

The flagellum is a more or less uniformly-staining filament. It varies in length. Trypanosomes of equal length may have unequal flagella (figs. 129 and 131). In round and globular organisms it is very short (fig. 120). In some it runs along the border of the body before becoming free (fig. 126). A few globular forms have a long flagellum (fig. 125).

#### Undulating membrane:

The undulating membrane in the cultural forms is short. In the majority of crithidia it is attached to

the convex edge of the curved body (figs. 127 and 156). The free border is longer than the attached one (fig. 127), hence it is thrown into folds. One to three folds are generally present. Attenuated forms have one or two short folds of undulating membrane. Stumpy broader and dividing forms have no membrane.

(d) Division:

The division forms of the trypanosome of the Mirror carp in all stages are relatively common in culture and show the details of division like the cultural forms of T. striati of Indian fish previously described.

Usually the various nuclear changes associated with the division of the nucleus are displayed best in coverglass impressions from plate cultures when stained with iron haematoxylin stain. On the other hand in fluid medium cultures, it is very difficult to see any details of nuclear development. Curiously enough the contrary is the case in the trypanosome cultures of the Mirror carp where the writer has seen all the changes in the nucleus best in fluid culture.

Changes in the parabasal body:

Changes in the parabasal body of this organism are almost identical with those described in T. striati



but here a marked line or cord between the two new parabasal bodies is to be observed (fig. 117).

Changes in the nucleus:

Before the division of the nucleus, it becomes swollen and loose in structure and its chromatin breaks up. At this time, the series of changes in the nucleus which occur when a parasite undergoes division are far from simple because the changes of the chromatinic substance take place before the commencement of the karyosomic division as the chromatin granules themselves divide into two groups. After this process has taken place the general division occurs by the elongation of the nucleus associated with an elongation of the karyosome. Eventually two nuclei similar to the original one are produced.

Division of the cytoplasm:

Division of the cytoplasmic body follows on completion of the division of the parabasal body and nucleus and the formation of a new flagellum. The separation commences at the anterior end and extends backwards. The two individuals then remain attached by their posterior ends for some time until final

separation takes place. The newly formed flagellates are unequal (fig. 154).

Sequence of events in division and  
types of parasites in culture.

As far as the sequence of events in division and the types of parasites in culture are concerned, it can be said briefly that they show practically no difference from the description given of cultural forms of the trypanosome of Ophicephalus striatus.

COMPARATIVE STUDY OF CULTURAL FORMS OF  
T. STRIATI . AND THE TRYPANOSOME OF  
THE MIRROR CARP.

As far as the writer could ascertain from the literature, the question of comparative study between the cultural forms of trypanosomes of fish cultivated in NNN medium has not so far occupied the attention of investigators.

The culture of goldfish trypanosomes has been obtained by Thomson (1908) but he could only maintain it for forty three days. He described the various developmental changes in detail at different set periods of time but was unable to compare these with those of other fish trypanosome cultures.



Tanabe (1924) succeeded in getting a culture of the trypanosome of the loach of Japanese waters in Ponselle's medium but he neither described its changes nor compared it with cultures of other fish trypanosomes.

The writer has obtained cultures, for the first time, of trypanosomes of Indian as well as European freshwater fish belonging to different families in the medium recommended by Shortt. After having described the life cycles of both organisms in cultures, in detail, it is thought that they should be compared in the light of the various developmental changes.

#### Differences of cultivation:

The culture of the Indian trypanosome was obtained at 24°C. temperature three weeks after introducing infected fish blood into the medium mentioned before. The culture was found to be heavy and subcultures were equally so. The European trypanosome culture was obtained after four weeks incubation at 16°C. and growth was moderate. Subcultures show rich growth.

#### Structure of the organism in fluid and solid medium:

The nuclear changes of T. striati, connected with the division of the nucleus show very clearly in coverslip

impressions from plate cultures but the cultures in fluid do not show the above mentioned details clearly. On the other hand, the trypanosome cultures from the Mirror carp are found to be the reverse as regards clarity in solid and liquid media.

The morphological characters of both the parasites in question have been described individually and separately in detail in the foregoing pages. It may be convenient now to summarize the comparative characteristic features in tabular form. They are as follows:

P.T.O.



No. Detail of characteristic features.	<u>T. striati</u> from (Indian fish)	<u>T. winchii</u> from (European fish).
1. Shape.	1. Elongate or spear headed in fluid. 2. Stumpy and broad with various shapes in plate.	1. Elongated, spear headed and broad in fluid. 2. Stumpy, globular and broad with different shapes.
2. Cytoplasm.	1. Finely granular with a few volutin granules stained in Giemsa. 2. Cleft like streak is present in a few forms. 3. In dividing forms one is more lightly stained than the other, i.e. differentiation between daughter and mother forms. 4. Rhizoplast not so clear.	1. Finely granular with many volutin granules stained with Giemsa. 2. No streak has been observed so far. 3. No such differentiation present in the cytoplasm of dividing forms. 4. Rhizoplast in many is clear as dark line with iron haematoxylin.
3. Nucleus	1. Nucleus small. 2. It is not situated on the posterior end. 3. Karyosome small and on more extraction of stain it is dot like. 4. Centrodosome of various types is present. 5. Division of karyosome with centrodosome is seen.	1. Nucleus comparatively large. 2. In some it lies at the posterior extremity. 3. Karyosome large on extraction. 4. No centrodosome observed. 5. Division of karyosome without centrodosome seen in a few forms.
4. Parabasal body.	1. Generally elongated or rod like, a few forms with dot like parabasal. 2. Small. 3. No cord between the dividing parabasal bodies.	1. A few elongated and round or oval. 2. Comparatively large. 3. Cord seen connecting the dividing parabasal.
5. Flagellum.	1. Relatively longer.	1. Relatively short.
6. Dimensions.	Length 4.5 $\mu$ to 40.0 $\mu$ Breadth 2.0 $\mu$ to 8.5 $\mu$ Nucleus 1.5 $\mu$ to 3.75 $\mu$ Parabasal .5 $\mu$ to 1.0 $\mu$ Flagellum 5.0 $\mu$ to 10.0 $\mu$	Length 3.2 $\mu$ to 18.0 $\mu$ Breadth 1.5 $\mu$ to 7.0 $\mu$ Nucleus 1.5 $\mu$ to 3.0 $\mu$ Parabasal .5 $\mu$ to 1.0 $\mu$ Flagellum 2.5 $\mu$ to 8.0 $\mu$



While there are some rare and exceptional differences between the two organisms there are general resemblances and affinities in their general shape and position of parabasal bodies, comparatively short free flagellum and straight membranes. Besides this nearly all the developmental stages are identical and the succession of forms are regular in both the cultural forms. This can be illustrated by the following table:-

Parasite.	Stages of the flagellates.			
	1	2	3	4
<u>T. striati</u>	Trypanosome primary culture.	Trypanomorphic crithidia.	Crithidia.	Metacyclic trypanosome
<u>T. winchii</u>	do	do	do	do

A COMPARISON OF THE CYCLES OF DEVELOPMENT  
OF T. WINCHII IN ARTIFICIAL CULTURE AND  
T. DANILEWSKYI IN THE INVERTEBRATE HOST.

It has been noted that in cultures, trypanosomes assume forms similar to their stages of development in the leech host. This kind of change undergone by the trypanosomes in cultures was regarded as an imitation of their invertebrate phase. Although there can hardly be any doubt that there is a parallelism in the stages of



trypanosomes in culture and in the vector host yet there are some phases in the latter which the writer has not seen in cultures. One such is leptomonas forms which are observed four days after feeding the clean young leech on infected fish. The comparison of the cycles of development is as follows:

Cycles of development	Stages of the flagellates.			
	1	2	3	4
1. In the culture.	Trypanosome (primary culture)	Trypanomorphic crithidia	Crithidia	Metacyclic crithidia
2. In the leech.	do	do	do	do

AN EXPERIMENTAL STUDY OF T. DANILEWSKYI  
AND ITS TRANSMISSION BY THE LEECH  
(HEMICLEPSIS MARGINATA).

Morphology and life cycle of the trypanosome in the leech.

It is known that in nature the transmission of trypanosomes and trypanoplasms from the blood of one fish to another is effected by the blood sucking leeches. This was noted and described by investigators who had examined and found various developmental changes of trypanosomes

in different species of leech.

Leydig (1857) recorded for the first time the presence of flagellates in the crop of two species of leech namely, Pontobdella and Piscicola which had fed on fish. Forty three years after (1901) Doflein (Germany) stated that the leeches might be the vectors of the fish trypanosomes. Brumpt (1904, 1905, 1906 and 1906a) noted the development of trypanosomes in large numbers in the crop of Hemiclepsia marginata which had fed on infected fish and in 1905 he succeeded in infecting carp and bullheads by trypanosome infected leeches. He also described the development of T. granulorum of the eel in the leech in question which was kept in the incubator at 26°C. temperature. He then stated that after division had taken place in the crop the trypanosomes migrated towards the anterior part of the body and swarmed in the proboscis sheath. Kesselitz (1904 & 1906) transmitted the trypanosomes of carp, pike and tench by the agency of Piscicola geometra. He also gave a most complete study of the trypanosome cycle in the same leech, and described conjugation of forms in the crop which still entirely lacks confirmation. Robertson (1911) has studied the development of trypanosomes and trypanoplasmas of the goldfish in the leech Hemiclepsia marginata.



She noted that rapid multiplication of forms took place in the crop and slender comma-shaped forms were produced. After some days these invaded the proboscis sheath and from the 10th day onwards the leech was infective. Tanabe (1924) has also given a description of the development of the trypanosomes and trypanoplasms of the Japanese loach in a leech.

#### Materials and methods.

Fishes Cyprinus carpio were obtained from Stambridge Fisheries, Essex. The blood from the caudal fin was examined and showed light infection of Trypanosoma danilewskyi. The leeches, Hemiclepsis, were collected from the pond at Queensberry Lodge in the garden of the Lister Institute at Elstree. This address was kindly given to the writer by M. Robertson when I consulted her in this matter. Piscicola were collected and sent to the writer by the Director, Freshwater Biological Association, Ambleside.

Out of a batch of Hemiclepsis collected from the Institute in question and brought to the laboratory at Winches Farm, St. Albans, three leeches were found with small young ones attached to them on the ventral surface of their bodies. These three were kept in a

separate beaker with some pond water and weed. The young leeches left their mothers four days later and attached themselves to the weed. The other leeches were put in another beaker and it was placed in the incubator, at a constant temperature of 24°C. so that they might produce cocoons. Young were seen 15 days later.

Piscicola were also kept in a small aquarium with weed at room temperature. They produced cocoons on the surface of the glass and on weed, and broods were seen a month later. Young clean Piscicola were put on fish; they sucked a very small quantity of blood and did not survive more than a day or two. Many were even unable to suck the blood at all. Several batches of these young Piscicola were tried in this way at different intervals with negative results.

In contrast with Piscicola, the young Hemiclepsis took hold of the fins and scales of the fish very readily when they were given the opportunity and sucked the blood for four to five hours. After feeding, the leeches were picked up gently with the help of forceps by holding the fish out of water with a clean cloth and were then kept in a wide mouthed bottle with water and weed.

The examination of these was carried out at various set periods of time. The leeches for microscopical



examination were first chloroformed and after pinning on the dissecting board, were opened with a fine sharp scalpel from the dorsal surface of the bodies to let the blood flow out. One drop was taken with a fine pipette on a clean slide with .9 per cent saline solution on it. After examining the fluid containing parasites, it was fixed by exposure to osmic acid vapour for fifteen seconds, then immediately smeared out with another clean slide, fixed in methanol for five minutes and stained with Giemsa stain. Some films were fixed in Schaudinn's and Flemming fixatives and stained with Heidenhain iron haematoxylin. The early stages of development were found in the blood in the crop already stated above and this was made into films by diluting it with .9 per cent saline as the content of the crop of the leech was found to be viscous. The trypanosomes and trypanoplasms in all their stages of development were found in the crop.

All drawings were made with the camera lucida with the same magnification as in the case of T. striati of an Indian freshwater fish.

#### General observations:

The small slender young Hemiclepais marginata after a full feed for about four to five hours has a

very characteristic swollen appearance, the body being greatly distended by the ingested blood. It becomes three times as large as before feeding. In this condition the leech is inactive and sluggish and remains motionless with the body contracted.

At the end of the first three hours after the meal of the leech, the trypanosomes appear to be decreased in size, measuring  $17.75\ \mu$  to  $20.75\ \mu$  in length;  $2.25\ \mu$  in breadth and free flagellum  $2.5\ \mu$ . The initial change seems to consist in a concentration of the cytoplasm and a bringing close together of cytoplasmic contents. The posterior two thirds of the parasite become expanded while the anterior third remains normal. The parabasal body becomes round (fig. 168) or elongated (fig. 169) and appears to be moved a little forward from its original position. The cytoplasmic granules are comparatively small in size, fewer in number and scattered throughout the whole body; the undulating membrane and free flagellum are shortened (figs. 168 & 169).

Five hours:- At this period there seems much alteration especially in the posterior two thirds of the body. A few cytoplasmic granules are present and the undulating membrane is also present (fig. 170). No divisional forms are met with. The measurements of these forms are as follows:-



Length.	..	..	13.0 $\mu$
Breadth.	..	..	2.25 $\mu$
Free flagellum.	..		2.0 $\mu$
Nucleus.	..	..	2.0 X 1.5 $\mu$

One day:- The blood at this period is somewhat viscous and in it both individuals and dividing forms of trypanosomes are present. An examination in the fresh state and in stained preparations reveals no trace of leptomonad types. The parasite assumes a somewhat stumpy and tadpole like appearance, with the parabasal body anterior or posterior to or on either side of the nucleus. That is to say the product of division is a crithidial form (figs. 171, 172 & 175). They are 8.5 to 11.5  $\mu$  long; 3.0 to 4.5  $\mu$  broad and the free flagellum is 1.5 to 2.5  $\mu$ . The forms showing division are only a few stumpy forms and there is hardly any distinction in size between daughter and mother individuals (figs. 173 & 174), but by close observation one may appear larger which may be considered as the mother form. A few are elongate and their bodies at their anterior ends taper. Both mother and daughter individuals are crithidial forms throughout the whole process of their division. No leptomonas stage has so far been seen. In stumpy dividing forms the nucleus lies in the centre of the body with the parabasal body in front or on either side of it. The free flagellum is

short; the undulating membrane is absent or, if present, has only one or two small folds.

In a microscopical section of an infected leech the individual and dividing flagellates are seen to be disseminated throughout the whole crop.

Two days:- The blood of the crop is viscid and on examination of it in the microscope the flagellates appear to be the daughter individuals of the second division i.e., the third generation of the originally ingested parasites are more numerous than on the first day. They are actively motile, moving in a straight manner unlike the trypanosomes inhabiting fish blood. In shape apparently there seems no change in crithidial form and in structure namely they are spear-headed with or without folds of undulating membrane and a short free flagellum (figs. 176 & 177). Dividing forms are also like the type of the 1st. day (fig. 178). They are  $8.5\ \mu$  to  $12.0\ \mu$  by  $4.6\ \mu$  and free flagellum  $2.0\ \mu$  to  $2.5\ \mu$ .

Three days:- At this period the blood is still thick and many red blood corpuscles are distorted. In fresh preparations, the flagellates move as those of 12 and 24 hours old. In stained films they are found to represent the three following types:-

I. Apparently unaltered crithidial forms which seem to have the power of multiplying freely and constantly



and resemble those seen on the 1st. and 2nd. days after the initial feed (figs. 179 & 181). The measurements are as follows:

Length.	9.5 $\mu$ to 12.5 $\mu$
Breadth.	2.5 $\mu$ to 3.5 $\mu$
Free flagellum.	2.0 $\mu$ to 2.5 $\mu$

II. Stumpy forms, a few, showing relatively large nuclei, short free flagella and without undulating membranes. They are 9.0  $\mu$  long; 5.0  $\mu$  broad and the flagellum is 1.5  $\mu$  (fig. 180).

III. More numerous longer forms with narrow pointed posterior and tapered anterior ends. These forms too usually have no well developed flagella and undulating membranes. The length is 10.5  $\mu$  to 14.0  $\mu$ ; breadth 2.5  $\mu$  to 3.0  $\mu$ ; and free flagellum 1.5  $\mu$  to 2.0  $\mu$ . It is possible that most of the single individuals of this type seen are the product of recent division and are themselves preparing for the initial stages of that process. Division forms are common, some of which show dividing nuclei connected with a fine cord (figs. 183, 184 & 185).

Four days: The contents of the crop of the leech are found to be less glutinous, with a few red blood corpuscles. Examination in the fresh state shows

enormous numbers of all the flagellated forms previously present, especially of types I and II. Many slender forms (figs. 188 & 189), some crithidial and a few stumpy forms are present. In some thin and elongated individuals the nuclei are situated at the posterior end and the parabasal bodies in the anterior half of the parasite. This condition is seen only 96 hours after the initial feed. They represent leptomonas forms (figs. 187, 190 & 191). Multiplication at this period is active and the dividing forms are either stumpy or elongated (figs. 192 & 193). They are 11.0  $\mu$  to 17.0  $\mu$  long; 1.5  $\mu$  to 3.0  $\mu$  broad and free flagellum 3.75  $\mu$  to 4.0  $\mu$ .

Five days:- In five days the number of the parasites is still greater. Fresh preparations show the parasites exhibiting the same movements as those of the fourth day. In stained films the type II flagellates become rather long (figs. 201 & 204). Type I still survives at this period but appears somewhat small in size. No leptomonas-like forms are present. A few dividing forms are met with (fig. 198).

Six days:- The features presented in fresh and stained films under the microscope are nearly the same as those of the fifth day. All the three types are



present though they are small in size. Slender crithidial forms appear more attenuated with the posterior ends either pointed or somewhat blunt (figs. 205 & 208). Flagellates stained with Giemsa are correspondingly larger and bulkier with volutin granules, vacuoles and small folds of undulating membrane. Attenuated forms possess parabasal bodies at the posterior end of the bodies though not at the extremity (figs. 209-210). Thus they appear in structure like the original normal blood-dwelling type. The measurements are as follows;

Length.	..	..	18.0 $\mu$ to 24.25 $\mu$
Breadth.	..	..	1.0 $\mu$ to 1.75 $\mu$
Free flagellum.	..	..	2.0 $\mu$ to 3.0 $\mu$

A few dividing forms are present (fig. 211).

Seven days:- The development appears to have to come to a close. Numbers of type I, though still remaining, are seen gradually lengthening to the so-called long attenuated form (fig. 216). In the slides prepared by Giemsa stain there are seen individuals containing volutin granules scattered throughout the whole body, elongated massive nuclei and comparatively large parabasal bodies. The free flagella compared to the size of the bodies are very

short. In some forms the flagella end at the anterior end of the bodies (figs. 217 & 218). All these forms, unlike stumpy and crithidial individuals, are sinuous and tapered. They are 20.5  $\mu$  long; 1.0  $\mu$  to 1.5  $\mu$  broad, free flagellum 1.5  $\mu$  to 2.0  $\mu$ ; and nucleus 2.5  $\mu$  to 6.0  $\mu$ .

Eight days: Almost all parasites have grown into very long, slender and attenuated forms (figs. 222 & 226). The posterior ends are comparatively sharp and pointed. As for the position of the organelles, the nuclei generally speaking are situated in the anterior half of the bodies, but in a few they are central or slightly posterior. The parabasal bodies lie posteriorly about the junction of the posterior third and anterior two thirds of the body of the parasite, either very near to the nuclei or some distance behind. The undulating membrane is present, the free flagellum is either very short or terminates just on the anterior end of the body. The flagellates types I and II are not present probably because they have been converted into long bodies and many small but attenuated forms are to be seen. In fresh preparations these forms move slowly in a straight line with the free flagellum forwards. Flagellates stained with Giemsa stain show volutin granules in the cytoplasm.



Nine days: The flagellates are nearly the same as at eight days but are correspondingly longer with developed undulating membrane, and a short free flagellum. Several small forms also can be seen which, as mentioned before, may have been types I and II (fig. 227). The length of the body is  $51.0\ \mu$ ; breadth  $1.0\ \mu$  and free flagellum  $1.5\ \mu$ .

Ten days: At this period the forms are still slender and attenuated with elongated nuclei and somewhat longer flagella. The position of the parabasal body is the same, as in the eighth day form. (figs. 229 & 230).

#### General review of the result of the experiment.

So far the writer has dealt with the description of findings in T. danilewskyi of the carp in the crop of the leech Hemiclepsis marginata in special reference to development after specific time periods. Now the various developmental changes of the parasite which take place in the leech will be described.

The flagellates that are taken into the crop of the leech when it feeds on a fish do not show any change either in shape or structure for two hours. After three hours the shape is changed and the size is decreased,

that is to say, the posterior two thirds of the body becomes swollen and the parasite appears tadpole-like in appearance. Much contraction of the body takes place five hours later, but no trace of division of any part of the body is seen. Nine hours after feeding, they are frequently to be seen in division. In two to five days the whole crop is filled with the trypanosomes in *Fig. 163* continuous active multiplication by division. During this period the parasite shows three types:-

Type I - Crithidial forms which seem to have the power of dividing frequently (figs. 163, 164, 176, 177, 181 & 188).

Type II - Stumpy forms which also show division (figs. 180, 182 & 201).

Type III - The product of types No. I and II which gradually become elongated and more and more slender in appearance (figs. 191, 203, 206, 207, 222 & 227).

The types I and II in question appear in one to three days after first feeding and continue to exist till six days. Meanwhile, some of them increase in size and begin to lengthen out. On the 4th and 5th day, the result of multiplication is really amazing and the crop is filled with innumerable parasites of all three types. The slender creatures swarm near the periphery of the



anterior branch<sup>es</sup> of the crop. This give evidence of migration of the flagellates from the posterior branches of the crop towards the anterior and eventually to the proboscis sheath. On the sixth day very few dividing forms are seen and the individuals instead of multiplying get more and more attenuated as time goes on until they reach a normal length and become what is called by Robertson "inoculative forms". From seven days onwards no dividing individuals are seen in any part of the crop. The flagellates continue to pass to the anterior end of the body into the proboscis sheath in great numbers and stay in this situation. After ten days this can be shown experimentally by cutting the leech into two parts, the anterior part being one third of the body, the other two thirds and by examining the contents from both with a drop of saline.

The question arises as to whether the forms that are capable of multiplying, display any distinctive distribution in the crop governed by the individual predilection of the various forms for special sites in the crop of the leech. So far as the writer's experience shows the dividing forms have no special selective distribution but occur indiscriminately with stumpy and long attenuated types.

The slender attenuated forms always travel in a straight line with the anterior free flagellum forwards while broad and stumpy types move round and round or in an irregular manner. So it is possible that the former individuals move towards the anterior end first and the latter two types later, and only after having assumed the shape of the inoculative types and their migration into the proboscis sheath is due to the response to some chemical or physical stimulus.

No conjugation between individuals of any type and no division in the inoculative forms have been so far seen in fresh or stained films by the writer.

The life history of T. danilewskyi which will be described subsequently in the leech is a history of intensely active multiplication resulting in an increasingly large output of all the stages and types already mentioned until the whole crop becomes a seething mass of active flagellates with indication of concentration of the parasites in the proboscis sheath. This is the stage which is observed by the 5th, 6th and 7th days after the initial feed of the leech. The chief point now to be considered is the relative numbers of these forms and the question as to whether they show any conspicuous distribution in the crop governed by the



individual liking of the various types for special sites in the crop of the host. The relative numbers of the various forms can only be accurately assessed by the counting of the total flagellate contents of leeches.

If the infection be traced it will become apparent that in almost all leeches, the development of flagellates is at first in the whole crop. As time proceeds, there is a continuous extension forwards of long forms from the massive growth in the crop. These are unable to go backwards so they settle in the proboscis sheath. They are regurgitated into fish when the leech is given a second feed. It has been noted by Robertson that the attenuated forms do not divide and form new masses anteriorly to the main parent mass. The same impression is gathered by the writer, namely that they are not as a rule dividing forms. The probability is that they are the end products of division in the leech. They develop into trypanosome forms only in the blood of fish and then are ready to divide when they happen to come again into the leech.

A careful consideration of the difference in the types of the flagellate present in the preparations made from the crop leads to a comparison of these findings with the conditions present in the artificial culture

of the trypanosome of the Mirror carp in NNN medium. The writer thinks as the result of careful observations that the conditions present in the crop of the leech are identical, in so far as the different environment permits, with those obtaining in artificial cultures in NNN medium modified by Shortt. The presence of innumerable free-swimming slender attenuated flagellates and the development of other forms has its analogy in the fact that the same products of development are present in the fluid part of NNN medium. Also the development of stumpy and broad forms, the products of continuous and active multiplication in the crop has its analogy in the same type of flagellate which grows on the dry surface of NNN plates. Therefore these observations make it clear that the artificial culture in NNN medium is an accurate recapitulation of the natural course of development in the leech.

From the foregoing description, it is evident that the life-cycle of T. danilewskyi is completed in two animals namely the carp and the leech Hemiclepsia. As described by previous workers, in the blood of the carp one part of the cycle is passed in which the predominant type is a trypanosome and the second part is completed in the crop of Hemiclepsia marginata where



the predominant form is a crithidia.

In the blood there are found the large and small trypanosomes mentioned above. These differences of size are due to the fact that the slender attenuated forms when settled in the proboscis sheath of the leech are of different dimensions. This same variation is apparent in the blood of the fish where no dividing forms have been seen.

The cycle of the trypanosome in Hemiclepsia marginata always takes place in the crop and the parasites do not pass further back than the crop. The characteristic form of this part of the life-history is a crithidial type in which the parabasal body is situated in front of or just behind or close beside the nucleus. The body, as noted before, is shorter, broader, more pear-shaped and tadpole-like than in the trypanosome. The undulating membrane is undeveloped or rudimentary. The body is held straight and rigid in progression instead of being flexible and sinuous. The free flagellum is very short. These crithidial forms generally differ in shape and size from each other but there is a slight difference in the position of the structural parts, namely nucleus and parabasal body. This is type No. I.

Meanwhile, the II type which is broad and stumpy in appearance is also produced. Eventually these two

crithidia phase types gradually become slender to long attenuated forms and pass forwards to the proboscis sheath which becomes infected.

TRYPANOPLASMA WILLOUGHBII n.sp. OF THE CHAR  
(SALVELINUS WILLOUGHBII AND ITS CULTURE)

Trypanoplasma willoughbii n.sp. has been found in the blood of the Windermere Char, Salvelinus willoughbii caught from Lake Windermere, Ambleside. The work was done in the laboratory of the Freshwater Biological Association.

So far as the writer knows, the occurrence of a trypanoplasma in the blood of the char has not been noted before, either in Great Britain or in other European countries though some investigators have found it in fishes such as trout and pike. The trypanoplasms were in small numbers. In the fresh blood, the parasite moves quickly and swims like a serpent, occasionally coiling up its body and repeating this motion repeatedly. In living preparations two forms, large and small, are seen. In stained preparations they exhibit this polymorphism. There are three types small, medium and large. The large forms, full grown individuals, are in greater numbers than the small and medium ones.



Movements of Trypanoplasma willoughbi:-

The writer has watched carefully for a considerable time, the movement of this trypanoplasma in fresh blood placed on the slide. It travels frequently with the anterior free flagellum forwards but occasionally can go backwards when it finds any obstacle in its way. While moving backwards, it seems to receive help from the posterior flagellum which runs along the edge of the undulating membrane. When the blood is fluid the movement is very active, especially among the small forms. Sometimes the organism coils up its body and wriggles but less so than the trypanosomes of fish. The large forms have a more sluggish movement, and get slower still when the fluid begins to dry up. If a drop of saline or citrate saline is added the movement again becomes active as before.

So far as observed and watched by the writer, the locomotion of a trypanoplasma with the help of its flagella takes place as follows:-

A vibration occurs in the undulating membrane on one side of the body starting from the anterior end and passing to the posterior end. This is due to the posterior flagellum which moves constantly and allows the undulating membrane to vibrate. No sooner does this

wave terminate than the anterior free flagellum withdraws back and then suddenly flings forward forcibly. Then another vibration commences immediately from the other end in the undulating membrane and the anterior flagellum shoots forward as before. This happens repeatedly in quick succession and thus the body is pushed forwards. It seems that the posterior flagellum with the undulating membrane acts like the paddle of a boat and the anterior like a rudder.

The organism sometimes changes its direction by contraction of the body. During this movement, the nucleus and volutin granules inside the body move constantly. The parabasal body has not been seen in the living trypanoplasma though it has been observed in stained specimens.

#### Morphology of Trypanoplasma willoughbii:-

The body is elongated and less tapering. The anterior end is broader than the posterior (figs. 237 to 240) and both are bluntly rounded; the cytoplasm is not homogenous. It varies according to the size of the organism. A small form has less stainable cytoplasm while the large one stains densely. The cytoplasm contains several vacuoles of various sizes and shapes, which are



present generally in the anterior portion of the body. Large volutin granules are scattered throughout the whole body.

The nucleus is of various shapes. It is oval (fig. 237), bean like (figs. 238 & 243) or irregularly shaped (figs. 239 to 242) and lies along the convex side just behind the anterior third of the body. In some it is in the posterior half (fig. 238). It is a vesicular structure with a distinct karyosome either connected to the nuclear membrane (fig. 237) or unconnected in the centre (figs. 241 & 242). A few chromatoid bodies are also present in the nucleus. The parabasal body is very large and elongated, and is situated at the anterior end along the concave side of the body and in some is divided into three parts (fig. 238). It is a compact mass which stains deeply, but some are found loose in structure (figs. 237, 239 & 242). Closely anterior to the parabasal body are two blepharoplasts (figs. 238) from which arise the two flagella. One of these ends freely at the anterior end as soon as it arises from the blepharoplast, while the other runs posteriorly ending freely near the posterior end. The undulating membrane in some is narrow (figs. 237, 238 & 240) but occasionally it is broad (figs. 240 & 241). Volutin granules are present in the

undulating membrane. The dimensions of the organisms are as follows:-

	<u>Small</u>	<u>Intermediate</u>	<u>Large.</u>
Length of the body. ..	20.0 $\mu$	25.0 $\mu$	26.5 $\mu$ to 36.5 $\mu$
Breadth of the body. ..	6.5 $\mu$	5.0 $\mu$	6.5 $\mu$ to 8.0 $\mu$
Length of the nucleus..	3.75 $\mu$	4.0 $\mu$	4.5 $\mu$ to 7.0 $\mu$
Breadth of the nucleus.	1.75 $\mu$	2.0 $\mu$	2.0 $\mu$ to 3.0 $\mu$
Length of the parabasal body. ... ..	3.0 $\mu$	4.5 $\mu$	7.0 $\mu$
Length of free anterior flagellum.. ... ..	16.0 $\mu$	14.0 $\mu$	16.5 $\mu$ to 19.5 $\mu$
Length of free posterior flagellum. ... ..	4.5 $\mu$	3.0 $\mu$	2.0 $\mu$ to 6.0 $\mu$

Systematic position:

Trypanoplasma willoughbi from the Windermere Char is the first record of this species.

<sup>y</sup>  
Kesselitz (1906) who studied trypanoplasms from certain freshwater fishes regarded them as all belonging to the one species Trypanoplasma borreli of the Rudd described by Laveran & Mesnil (1901). Other investigators, on the other hand, thought that if all these flagellates were the same form, the dimensions, position of the nucleus and parabasal body, long and short free flagella and undulating membrane should correspond closely in form and



dimensions. As, however there were considerable differences they gave specific names to the trypanoplasms inhabiting different fish.

Tp. willoughbii agrees somewhat in general shape and structure with Tp. borreli and Tp. cyprini (Plehn 1903), but the situation of the nucleus and parabasal body and the dimensions are different. The body of Tp. borreli is broad at the anterior end and tapers at the posterior. Both anterior and posterior free flagella are equal in length. The dimensions are 20  $\mu$  long and 3  $\mu$  to 4.0  $\mu$  broad. Tp. willoughbii is identical at the anterior and posterior ends of the body (in some the posterior end is somewhat tapering like that of Tp. borreli). The anterior flagellum is four times as long as the posterior one. The parasite is polymorphic the small form being 20.0  $\mu$  long and the large 36.5  $\mu$ .

Tp. gurneyorum has been described as a new species by Minchin (1909) from Esox lucius (pike). He put it under two forms 'ordinary and large'. Its shape is somewhat trypanosome-like and the large form is broad and stumpy. The nucleus is near the middle of the body and the anterior free flagellum is of moderate length. Dimensions are not given. These characters differ from those of Tp. willoughbii.

Tp. keysselitz<sup>y</sup>i was noted by Keysselitz. Minchin having described it in detail proposed to name the parasite of the tench Tp. keysselitz<sup>y</sup>i in honour of Dr. Keysselitz who was the first to observe it. It is found in two forms small and large. The posterior flagellum of the small form is long; the nucleus is small and situated a short way behind the parabasal body; the parabasal body is very much elongated. No dimensions are given. Whereas the posterior flagellum of the small form of Tp. willoughbii is very short, the nucleus is larger and generally situated at a point opposite the parabasal body.

Tp. abramidis from the Bream was discovered by Brumpt (1906). The body is rather slender and not granular. The anterior free flagellum is fairly long and the posterior moderately so. The nucleus is about on a level with the hinder end of the parabasal body. No large form has been found by Brumpt, Keysselitz and Minchin. It is 30.0  $\mu$  long; the anterior free flagellum is about 15.0  $\mu$  and the posterior 5.0  $\mu$  to 6.0  $\mu$  but Tp. willoughbii is not slender. It is granular.

Tp. varium from the loach is smaller than Tp. willoughbii. Leger (1904) found two forms and considered them as two different species.



Tp. barbi, found by Brumpt in the blood of Barbel, is 26.0  $\mu$  long; the anterior free flagellum is 18.0  $\mu$  and the posterior one 9.0  $\mu$ , whereas the intermediate form of Tp. willoughbii which is 26.0  $\mu$  long has its anterior free flagellum 14.0  $\mu$  and its posterior 3.0  $\mu$  long.

Tp. truttae from the trout occurred under one form. The anterior flagellum is 12.0  $\mu$  long which is smaller than that of Tp. willoughbii.

Still other trypanoplasms differ in structure and in dimensions from that of the Char.

The trypanoplasm of the Char seems to be a new species. It is therefore named Tp. willoughbii with characters as herein described.

The morphology and life-cycle of T<sub>p</sub>. willoughbii  
n.sp. in culture.

Laveran & Mesnil in 1901 investigated and studied for the first time the trypanoplasm in the blood of the Rudd Leuciscus erythrophthalmus to which they gave the specific name Tp. borreli. Since that date a number of similar forms have been found in different species of freshwater fish. The morphology, transmission and life cycle of the trypanoplasm in different species

of leeches, namely Hemicolepsis marginata, Piscicola geometra and Pontobdella muricata have also been described in detail by Brumpt (1904), Keyaseltz (1906), Robertson (1911) and Tanabe (1924). Ponselle (1913) studied the forms of trypanoplasm of the leech in culture. He was the first worker who attempted to culture these biflagellates in vitro. The medium used by him was 2 per cent agar in water without salt with which one volume of defibrinated rabbit blood was mixed. He then described briefly the cultural forms of the parasite.

Instead of the medium used by Ponselle the writer used Shortt's modification of NNN medium in which salt and glucose were added.

#### Culture from the blood:-

Ponselle in 1913 has reported for the first time good results in cultivation of Tp. varium of the loach. The culture was found 14 days after introducing the infected blood into the medium. He noted that the cultural form differed little from the blood flagellates. They showed the same position of nucleus, parabasal body and of flagella, the same size and the same movements. The undulating membrane was less developed. The division was binary longitudinal fission. No statement regarding subculture was made.



The writer cultivated Tp. willoughbii from the blood of the Char in NNN medium prepared according to the formula recommended by Shortt, under which conditions the trypanoplasms multiplied with great readiness. This gave the opportunity to study the course of development of this flagellate and the stages of evolution in culture.

The trypanoplasms appeared in culture after six to seven days. This fact in itself shows how easily the trypanoplasms adapt themselves to the medium. Their numbers reach their maximum about the 7th day of the culture after which they agglutinate or gather into masses.

The culture requires a low temperature, that is between  $14^{\circ}$  to  $16^{\circ}\text{C}$ . When grown at lower or higher temperatures the organisms develop slowly. Once the culture is established, it can be kept below  $14^{\circ}\text{C}$ . for a long time. On one occasion a tube was put in the refrigerator for a week and found positive when examined. Higher temperatures ( $20^{\circ}\text{C}$ .) stop the growth and eventually the parasites die within a week. It is interesting to note that the culture has been maintained by the writer for nearly two years during which sub-cultures have been made 90 times.

In order to study the life cycle, the writer

tried to cultivate the trypanoplasm on blood agar plates. Several attempts were made with rich culture fluid on solid medium but none gave positive results from two days to ten days.

Cultural characteristics of Tp. willoughbi

As mentioned before, very little difficulty is experienced in cultivating the trypanoplasm of the Char. The cultural forms become coarsely granular. Living forms are rarely to be seen after about four weeks although the writer, exceptionally met with them at the end of the sixth week. No circular colonies just above the fluid were observed as in the case of trypanosome culture. In the early primary culture obtained in six to seven days there are seen three types.

Type I. Free forms having the shape of normal blood dwelling flagellates, though comparatively more slender and smaller in size. They grow rapidly (figs. 247 & 250).

Type II. This is somewhat broader and bulkier. They appear comma shaped. They are wider near the anterior end and actively motile (figs. 262, 263 & 267).



Type III. This is a squat type which are small in numbers. The undulating membrane is broad (figs. 255 & 256).

The forms in subcultures do not differ from these except for minor differences. Old cultures show the three types mentioned above.

Shape.

The shape of Tp. Willoughbii in culture varies. Some are flattened and elongated (figs. 255 & 256), some broad and bulky (figs. 247 & 254) and some very stumpy (fig. 263). A few are spindle shaped. Dividing forms are finger like (fig. 283) and cone shaped (fig. 277), others irregular (figs. 270 & 278). Viewed under a high power of the microscope the body of the flagellate appears thick like a bladder filled with fluid with some granules floating in it. The posterior portion of some of the forms becomes swollen, appearing like a balloon, which the organism drags behind it (fig. 260). Cultural forms have neither twisted bodies nor any corkscrew like appearance.

Multiple fission forms may appear in subculture which look sun-flower like in appearance. This kind of agglutination is common in the first three or four days

of subculture.

Cytoplasm:-

The cytoplasm stains a very light blue with Giemsa stain. Sometimes it stains deeply and then it is very difficult to make out the nucleus. The most remarkable feature of these cultural forms is the presence of deeply staining large, round or rod shaped volutin granules in the cytoplasm. These are numerous in the posterior portion of the body. On the other hand, in iron haematoxylin preparations, the cytoplasm, like that of other flagellates, is finely reticular or vacuolar in structure. In stumpy forms a few large vacuoles are generally present posteriorly (fig. 268).

No other typical structure such as cleft like streaks, or obscure lines of chromatinic nature are present.

Nucleus:-

Almost all forms mentioned above have either a circular or oval nucleus. The nuclear membrane is not clear in Giemsa stain while it is marked in iron haematoxylin preparations. As far as the position of the nucleus is concerned, it is generally situated



very near the anterior portion of the body, either closely connected with parabasal body or a little distance behind or on either sides (figs. 254, 265, 267 & 283). In a few forms, the nucleus lies at the posterior end (figs. 249, 250 & 261) but in circular or globular organisms it is situated near the centre of the body. Nearly all daughter individuals in the multiple fission types have their nuclei in the centre with the parabasal bodies a some distance behind them (fig. 282).

The nucleus of dividing forms lies just beneath the parabasal (figs. 270, 272 & 274) but in some the already divided nuclei are at the posterior extremity and the parabasal at the anterior end (fig. 279).

The karyosome is relatively large and central and is very noticeable in iron haematoxylin preparations (figs. 266, 268 & 280). The karyosomic mass is not associated with any rod shaped structure or centrodesmose.

#### Parabasal body:-

The elongated and wedge shaped parabasal body is usually situated on the concave side of the body (figs. 249 to 252, 264 & 265) furthest from the undulating membrane and is remarkable for its great

length. It is typically cone-shaped, the anterior end being broader and the posterior tapering to a point. In globular forms, it assumes the shape of a round body (fig. 257) or may even be rectangular in other forms (fig. 258). With Giemsa stain the parabasal body appears very much larger than it does with iron haematoxylin, even two or three times as large.

#### Blepharoplast:-

Closely anterior to the parabasal body are two blepharoplasts from each of which arises a flagellum. Of these two, one appears larger and the other smaller; the former one gives rise to an axoneme and the anterior free flagellum while the latter, which is apparently absent due to identical staining of it and the cytoplasmic content produces the posterior flagellum. On the other hand, in iron haematoxylin stain both the blepharoplasts are to be seen distinctly.

#### Undulating membrane:-

In cultural forms the undulating membrane is not well developed. Type I shows a very narrow membrane (figs. 249 to 252), type II a very broad one while type III is intermediate in this respect.



Flagella:-

The flagella are very thin delicate and thread like filaments. The anterior free one is more than half the length of the body (figs. 251, 252, 264 & 268) or less (figs. 248 & 261) and the posterior free flagellum projects only a very short distance beyond the hinder end of the organism; in some flagellates it is longer, i.e., as long as the anterior flagellum (fig. 252). The writer has watched in fresh preparations the movements of all the forms. The globular forms were stationary; the elongated bodies showed slight movement, the undulating membranes of which are very short or even rudimentary. It seems that the active mortality of the trypanoplasm is due to the undulating membrane and that the free flagellum is incapable of causing a translatory movement of the body. In a few forms, the posterior flagellum is detached as a trailing flagellum giving the parasite a likeness to Bodo (fig. 261).

Division:-

The commonest method of division is binary fission which appears to be a comparatively simple type as compared with that of trypanosomes. Multiple fission produces a swarm of small trypanoplasms (fig.

282), some of which are slender and elongated in form. To give an account of the process of division is somewhat difficult as the writer, in spite of attempting to get suitable growth of dividing forms on solid medium plates, was unable to obtain them. As far as it has been observed it can be said that the first changes are seen in the blepharoplast, flagella and parabasal body; later, changes occur in the nucleus and finally division of the cytoplasm takes place. The division is initiated by the formation of a new flagellum from the blepharoplast from which the anterior free flagellum arises (fig. 251). Then the second new flagellum from the second blepharoplast which gives rise to the posterior flagellum follows (fig. 273). Next, the parabasal body becomes elongated and constricted at its centre (fig. 266) transversely and divides simply into two daughter parabasal bodies (fig. 275). Meanwhile the nucleus as a whole becomes constricted at the middle before dividing so that its division is usually succeeded or accompanied by that of the parabasal body. However, it has been observed that a parabasal body may divide in the absence of any division of the nucleus (fig. 269). Eventually longitudinal division of the cytoplasm to form two new organisms follows. Sometimes more than two new flagella



are produced before the separation of the parasites i.e., a precocious division of the organelles preceding a lagging division of the cytoplasm.

### DESCRIPTION OF FLAGELLATA FOUND IN THE BLOOD OF DIFFERENT SPECIES OF FISH.

In the following pages are given additional descriptions and information on a few already-noted species of trypanosomes and trypanoplasms which were found parasitic in the blood of some of the fishes of the British Isles as well as of India. Each flagellate described below was compared carefully as far as possible with known species with respect to their characters. They were also studied in life.

#### Materials and methods:-

Clarias batrachus and Saccobranchus fossilis were brought alive from India. As far as the British fish are concerned they were collected from various sources mentioned before, that is Bream from a gravel pit near St. Albans, Perch from Windermere and Essex, Minnow and Bullhead from Windermere.

Methods of staining and drawings employed are as noted before.

Trypanoplasma guernei (Brumpt)

Tp. guernei (Brumpt 1905) occurred in moderate numbers in the blood of six Bullheads, Cottus gobio, out of fifteen collected from Lake Windermere, Ambleside. The work on these was performed in the laboratory of the Freshwater Biological Association, Wray Castle, Windermere. The fish were very small, not more than two and a half inches in length, and hardly more than one or two drops of blood from the heart could be got for smears. Organ smears were made but without positive result.

In life, the trypanoplasma is less active than trypanosomes. Movement takes place by constant lashing of the anterior flagellum. Several large and one or two small forms have been seen.

The trypanoplasma is flattened and elongated with rounded anterior and somewhat pointed posterior end. The anterior end is four times as broad as the posterior one. It is sinuous and curved. The cytoplasm of large forms is dense and takes the stain deeply; it has numerous granules and vacuoles scattered throughout the whole body (figs. 286, 290 & 293) but they are fewer in small forms (fig. 287).

The nucleus varies in size and is irregular in



shape being elongated (figs. 285 & 287), triangular, (fig. 290), rectangular, (fig. 289) or spindle-shaped (fig. 292), and situated rather behind the anterior one fourth of the body near the convex border. In fig. 294 the nuclear membrane is shown to be very prominent and clear. No karyosome is observed but homogeneous chromatoid bodies are scattered in the nucleus. The parabasal body is an elongated or wedge-shaped body, situated at the anterior end along the concave side opposite to the nucleus. It is comparatively small and compact, and stained deeply with Giemsa. There is a blepharoplast close to or slightly separated from the parabasal body anteriorly (figs. 289, 291, 292 & 294).

The flagella are very distinct but finer than those of trypanosomes. The anterior free flagellum is longer than the posterior one, which passes antero-posteriorly to the posterior end of the body. The undulating membrane is narrow with three to five folds (figs. 285, 289, 290, 291 & 292). It has always been seen only on one side of the body differing in this respect from that of trypanosomes.

The measurements of Tp. guernei are as follows:-

Length of the body.	..	23.5 $\mu$ to 40.0 $\mu$
Breadth of the body.	..	2.5 $\mu$ to 7.5 $\mu$

Length of the anterior free  
flagellum. ... 17.0  $\mu$  to 21.0  $\mu$

Length of the posterior  
free flagellum. ... 2.5  $\mu$  to 20.0  $\mu$

Trypanoplasma cyprini (Plehn)

Trypanoplasma cyprini has been found scantily in the blood of four common carp (Cyprinus carpio) out of thirty caught from a gravel pit near Ehenley. Only large forms have been seen. The body is somewhat long and broad, often bent in a circle. It is soft with very delicate and thin periplast which is the reason why the body is always deformed on drying the blood smear. The form of the anterior extremity of the body shows variation which is perhaps due to movement of the trypanoplasma or due to deformations induced by fixation. Sometimes the anterior end appears bluntly rounded (figs. 296, 298 & 300).

The cytoplasm contains numerous volutin granules varying in size, and scattered throughout the body. A few vacuoles have been seen. The nucleus is elongated and of vesicular structure with clear karyosome. It has chromatoid granules. The position of the nucleus in the body varies. In figs. 296 & 300, it is either



very close to the parabasal body or at some distance (figs. 295 to 299). The parabasal body is wedge-shaped or elongated, tapering posteriorly and situated either at the extreme anterior end or in the middle of the body (fig. 299). It is a compact mass which stains deeply. A blepharoplast has not been observed in this parasite. The anterior flagellum is longer than the posterior which turns backwards and passes down the side of the body to the posterior end from which it projects freely. The undulating membrane is narrow. In figs. 296 & 297 it is rather broader than usual.

The dimensions of Tp. cyprini are as follows:-

Length of the body.	.. ..	18.5 $\mu$ to 37.5 $\mu$
Breadth of the body.	.. ..	5.5 $\mu$ to 12.5 $\mu$
Length of the nucleus.	.. ..	4.5 $\mu$ to 7.5 $\mu$
Breadth of the nucleus...	.. ..	2.0 $\mu$ to 4.0 $\mu$
Length of the parabasal body.	.. .. .	8.5 $\mu$ to 10.5 $\mu$
Breadth of the parabasal body.	.. .. .	0.7 $\mu$ to 1.0 $\mu$
Anterior free flagellum..	.. ..	14.0 $\mu$ to 27.5 $\mu$
Posterior free flagellum.	... ..	3.0 $\mu$ to 6.5 $\mu$

Trypanosoma danilewskyi var. batrachi

This trypanosome has been found in large numbers

in the blood of twenty and scanty in that of four out of thirty Clarias batrachus, an air-breathing freshwater fish of India, brought alive to London for investigation of protozoal parasites. Smears of the heart blood contained abundant trypanosomes, but comparatively few were seen in organ smears.

The trypanosome during life in fresh blood is transparent and moves very quickly with the free flagellum foremost, actively displacing the adjacent red blood corpuscles. Wriggling movements and twisting of the body into knots have been observed. A few stumpy forms have been seen in stained preparations.

As for morphological characters, the body is usually long, narrow and sinuous with both ends tapering, but the anterior flagellar end is more pointed than the posterior. The cytoplasm is granular with vacuoles varying in size. Volutin granules, often clumped together to form bigger masses stain deeply. The nucleus, situated in the posterior half of the body, is large, elongated and compact and occupies nearly the whole breadth of the body. In stumpy forms it is rather rectangular and loose in structure (figs. 307 & 308). The ends of the nucleus are surrounded by a clear halo. Karyosome and chromatin granules have not



been seen due to deep staining of the nucleus. The parabasal body situated at the posterior extremity is rather large and compact. It varies in shape, being oval, triangular or bean shaped. In fig. 308 it is surrounded by a vacuole. A blepharoplast has not been observed. The organism has a long flagellum and the undulating membrane bordering the body, makes three to six folds. No granules are found in the undulating membrane. Dividing forms were not present but one trypanosoma showed an early stage of division (fig. 305).

The following are the dimensions of the trypanosome:-

Length of the body.	.. ..	22.0 $\mu$ to 29.0 $\mu$
Breadth of the body.	... ..	1.5 $\mu$ to 3.5 $\mu$
Free flagullum.	.. ..	9.0 $\mu$ to 14.0 $\mu$
Length of the nucleus..	.. ..	2.0 $\mu$ to 3.0 $\mu$
Breadth of the nucleus.	.. ..	1.0 $\mu$ to 1.5 $\mu$
Width of undulating membrane...	.. ..	.75 $\mu$ to 1.5 $\mu$

Several trypanosomes have been found in the blood of freshwater fish belonging to the genus Clarias, but they are somewhat different from the trypanosome of Clarias batrachus. Montel (1905) discovered Trypanosoma

clariae in Clarias macrocephalus of Cochin China, but he neither gave any adequate description of the parasite nor its dimensions. In 1906 Dutton, Todd and Tobey found trypanosomes in two mud fish Clarias angolensis caught at Leopoldville in the Congo. They gave a full account of it along with dimensions. They saw three types of trypanosomes, small medium and large. All the forms were characterised by the possession of a large four-lobed granular blepharoplast, a long oval granular nucleus with karyosome, reticular body protoplasm with coarse granules and superficial longitudinal striations or myonemes.

The measurements were as follows:-

	<u>Small.</u>	<u>Medium.</u>	<u>Large.</u>
Body length. .. ..	22.5 $\mu$	31.5 $\mu$	49.0 $\mu$
Body breadth. .. ..	1.0 $\mu$	3.0 $\mu$	4.5 $\mu$
Free flagellum. .. ..	12.0 $\mu$	13.5 $\mu$	10.5 $\mu$
Nucleus... ..	3.0 $\mu$	3.7 $\mu$	4.5 $\mu$

While the trypanosome of Clarias batrachus is dimorphic having both large and stumpy forms the latter are very rare. The parabasal body is either round to oval or triangular or bean shaped. It is compact. The nucleus is large, elongated and compact but in stumpy forms chromatoid granules are present. The



flagellum is moderate in length. In dimensions it is also different from the organism mentioned by Dutton, Todd and Tobey. In addition the Indian trypanosome has no striations whatsoever either in stumpy forms or large forms.

In 1908, Wenyon merely reported the occurrence of trypanosomes in the blood of Clarias anguillaris which abounds in the Nile and in Lake Ambodi, Sudan. No description and measurements were given by him. Bouet (1909) found a trypanosome in the same fish mentioned by Wenyon and stated it was perhaps the same parasite as that of Clarias angolensis and therefore he gave no detailed account with dimensions.

Castellani and Willey during 1905-6 saw trypanosomes in Saccobranchus fossilis occurring in the Colombo Lake which they named T. saccobranchi without mentioning morphological characters and dimensions. They simply stated, "The caudal prolongation of T. saccobranchi was obsolescent, the centrosome being placed very far backwards much as in T. danilewskyi from the carp."

This trypanosome of Clarias batrachus recalls somewhat T. danilewskyi which has resemblances regarding the position, structure and characters of the parabasal

body, nucleus, narrow undulating membrane and dimensions. As far as the writer knows the host is new. It belongs to the family Siluridae whereas the carp belongs to the Cyprinidae. Therefore it is proposed to name the parasite Trypanosoma danilewskyi var. batrachi with characters as herein set forth.

Trypanosoma danilewskyi var. saccobranchi

The occurrence of trypanosomes in the blood of Saccobranchus fossilis was observed in four out of nine fish brought alive from India. Organ smears were found negative. No ectoparasites were observed.

When a drop of fresh blood from the heart is examined, the trypanosome shows very active wriggling movements like the trypanosomes of Clarias batrachus.

The body is fusiform, sinuous, pointed at the flagellar end and relatively blunt or irregular in shape at the other extremity. The cytoplasm is granular and massed in the anterior portion of the body and large vacuoles, while in the posterior portion the cytoplasm is clustered on both sides of the body with a tenuous intervening part. The compact nucleus, situated in the posterior half of the organism, is elongated or rod-like and stains a rather deep purple. It is parallel



to the axis of the body and adherent lengthwise to one side of the parasite, if it does not fill the whole width of it. In such cases, the non-adherent side of the nucleus stains diffusely and chromatoid granules are seen (fig. 312). Vacuoles are always present at the ends of the nucleus. The parabasal body is comparatively large, compact and varies in shape. It is either elongated, round or bar-like, and bilobed (fig. 314). In fig. 315 it is bean-shaped; a blepharoplast was not observed in most of the specimens but in one it could be seen and from it the flagellum seemed to arise. In fig. 311 the parabasal body is shown stained very lightly. The flagellum seems to originate directly from the parabasal body. It is very thick and prominent and runs along the border of an undulating membrane, but appears adherent to the body at some places. The free flagellum is nearly half the length of the body. The undulating membrane is thin and stains very lightly. No granules are found in the membrane. Six to nine folds of undulating membrane, with variable width, can be seen.

Dimensions of the organism are as follows:-

Length of the body. .. 27.0  $\mu$  to 32.0  $\mu$

Breadth of the body. .. 1.0  $\mu$  to 1.5  $\mu$

Free flagellum. . . . .	11.0 $\mu$ to 15.0 $\mu$
Length of the nucleus. . . . .	2.75 $\mu$ to 3.2 $\mu$
Breadth of the nucleus. . . . .	.7 $\mu$ to 1.0 $\mu$
Length of parabasal body. . . . .	.75 $\mu$ to 1.0 $\mu$

Trypanosoma saccobranchi was first discovered by Castellani and Willey in 1905-6 in Saccobranchus fossilis, a fish seven to eight inches in length occurring in the Colombo Lake. They named the parasite solely on the ground of its occurrence in Saccobranchus fossilis. They gave one figure only but with neither dimensions nor any description except the following:

"In Trypanosoma saccobranchi the caudal prolongation is obsolescent, the centrosome being placed very far backwards much as in Trypanosoma danilewskyi, Lav. & Mesn. from Cyprinus carpio."

The trypanosome which the writer has found resembles in many respects, except in dimensions, Trypanosoma danilewskyi in having a narrow undulating membrane, elongated nucleus surrounded by a narrow halo, relatively large and variously shaped parabasal body. It is therefore proposed to name it Trypanosoma danilewskyi var. saccobranchi.



Trypanosoma abramidis.

T. abramidis has been found in small numbers in the blood of the bream, Abramis brama out of a collection of thirty one supplied by the Verulum Angling Society of St. Albans from the gravel pit near the Colne river. The fish were from 6 to 9 inches in length. Organ smears gave negative results.

The trypanosome in life is transparent. It is very active and moves quickly. In stained preparations large forms have been found but one small type has also been seen (fig. 324).

The body is sinuous with both ends tapering, but the anterior end rather more acute than the posterior. It looks uniform in size while alive, but is variable in stained preparations. The cytoplasm is clear and contains small and large vacuoles. Several of these vacuoles coalesce together to form bigger patches either at the centre of the body or near its ends. Myonemes have not been detected. Volutin granules are present and distributed throughout the whole body. In some they are adherent together and are stained red and purple with Giemsa stain.

The nucleus is round to oval (figs. 322, 326, 328 & 329), pear shaped (fig. 325), or elongated

(figs. 318, 321 & 323). It is situated in the middle of the body occupying its whole width. The nuclear membrane is not clearly defined. The karyosome is not visible; granules of chromatin are present either on the inner surface (fig. 325) or scattered throughout the nucleus (figs. 322, 326 & 329). The parabasal body is comparatively large, round (figs. 325, 328 & 329) rather elongated (figs. 319, 321 & 323) or bean shaped (fig. 320) and is situated near the posterior extremity. It is compact and may be surrounded by a halo (fig. 328). In front of the parabasal body there is a distinct blepharoplast, but in some individuals it is not apparent.

The flagellum is long, nearly half the length of the body. The undulating membrane is narrow and always appears on the convex edges of the body. The free border is considerably longer than the attached one and hence four to six membrane folds are present.

The dimensions are:-

Posterior extremity of the body to posterior border of the nucleus.	.. 12 $\mu$ to 15.0 $\mu$
Posterior to the anterior border of nucleus (length of nucleus).	.. .. 3.0 $\mu$ to 3.5 $\mu$
Anterior border of nucleus to anterior extremity of body.	.. .. 9.5 $\mu$ to 15.0 $\mu$



Breadth of the body at its widest part. . . . .	1.5 $\mu$ to 3.0 $\mu$
Free flagellum. . . . .	12.5 $\mu$ to 15.0 $\mu$
Breadth of nucleus. . . . .	1.5 $\mu$ to 2.0 $\mu$
Length of parabasal body. . . . .	.75 $\mu$ to 1.0 $\mu$
Total length of the trypanosome including flagellum. . . . .	37.0 $\mu$ to 48.5 $\mu$

The trypanosome in the blood of the bream was first discovered by Laveran and Mesnil (1902) and the species was named Trypanosoma <sup>di</sup>abrami/s in 1904. They gave a very brief account of the parasite without much detail, or figures. Keysselitz (1904) observed the development of the trypanosome in the leech, Hemiclepsis. Minchin (1909) found a trypanosome in bream from Sutton Broad and stated briefly, "I have found a trypanosome very sparingly; three specimens altogether, one from one fish, two from another. The trypanosome is of large size and presents no character by which I can distinguish it morphologically from Trypanosoma tincae. I consider it very probably identical with this species, in which case the name tincae has priority over <sup>di</sup>abrami/s by one page; but in view of the scantiness of my observations, I refrain from taking the step of merging the two species into one".

In view of the above-mentioned facts, the writer, therefore, has outlined the characters of T. abrami<sup>di</sup> here for the first time though Minchin thought the parasite was similar to T. tincae which he has described.

To obtain cultures of the trypanosome, the writer introduced the blood from infected fish into NNN medium of Shortt's modification with full aseptic precautions but he was unable to obtain any culture. At the same time, the blood containing trypanosomes was kept between sterilized slide and coverslips. The edge of the coverslip was sealed with vaseline and the slide was then placed in the refrigerator in which the temperature varied between 15° and 18°C. The trypanosomes in the film had divided when examined four days later. The coverslip was removed and the smear stained with Giemsa stain.

In this film one trypanosome (fig. 327) was found unaltered, with its original characters, but the cultural or divided forms had changed from the adult types in shape and arrangement of parts (figs. 330, 331 & 333). The posterior two thirds had become short and swollen, the anterior third remained unaltered; the nucleus had become swollen and loose in structure; the parabasal body came up from the posterior extremity to



the centre of the body and lay very close to the nucleus; the undulating membrane was relatively shortened; the flagellum had become short. This form resembled a crithidia.

The measurements of the divided forms are as follows:-

Length of the body.	.. ..	13.0 $\mu$ to 24.0 $\mu$
Breadth of the body.	.. ..	3.5 $\mu$ to 6.5 $\mu$
Free flagellum.	.. ..	5.5 $\mu$ to 7.0 $\mu$

#### Trypanosoma percae (Brumpt)

T. percae was found in small numbers in the blood of two small perch out of a collection of ten each 4½" long, obtained from Windermere and Essex. The trypanosome can be distinguished by the characters which Minchin (1909) has described in detail with beautiful figures.

Both in fresh and stained preparations the organism was observed in two forms, medium and large. In life they are very actively motile, wriggling and twisting themselves up constantly.

As for morphological characters the body of T. percae is sinuous and spindle-shaped with both ends

tapering. The cytoplasm is granular and stains lightly. Very few vacuoles have been seen. Some free spaces are seen along the whole length of the body. A few irregular fairly large chromatoid granules are seen in the anterior half of the body (figs. 233 & 234). In preparations fixed with osmic acid vapour and stained with Giemsa one cannot see the chromatin granules due to the intensity of the stain. A wave-like undulating membrane is very distinct in this preparation while a short and narrow undulating membrane is present in dried preparations.

The nucleus is ovoid or elongated and is situated in the anterior one third of the body. A deeply stained spherical or ovoid karyosome (figs. 235 & 236) is present in the centre of the nucleus. It is large in size. Some chromatin granules are also seen. The comparatively large parabasal body, which is situated a short distance in front of the posterior extremity of the body, appears as a rounded mass. It is compact. A dot-like (figs. 233, 235 & 236) or rod-like (fig. 234) blepharoplast is seen just in front of the parabasal body. From this blepharoplast, the flagellum arises and runs towards the anterior end of the body, adherent either to the body or to the border



of the undulating membrane. The distinct undulating membrane is either narrow or wide.

The measurements of T. percae are as follows:-

Length of the body. . . . . 29.0  $\mu$  to 32.5  $\mu$

Breadth of the body. . . . . 1.5  $\mu$  to 2.5  $\mu$

Length of the nucleus. . . . . 2.5  $\mu$  to 3.0  $\mu$

Breadth of the nucleus. . . . . 1.0  $\mu$  to 2.5  $\mu$

Length of free flagellum. . . . . 9.5  $\mu$  to 12.0  $\mu$

Brumpt (1906) first noted T. percae in the European perch Perca fluviatilis. His brief description and dimensions of the parasite differ from those of the English trypanosome. He stated it was 57.0  $\mu$  in total length of which 16.0  $\mu$  was free flagellum, body breadth was 3.0  $\mu$ . Minchin (1909) described in detail T. percae in Perca fluviatilis found in Norfolk England. His description resembles the parasite found by the writer. Pantham, Porter and Richardson (1942) observed T. percae from Canadian fish. They noted it was 29.9  $\mu$  to 32.5  $\mu$  in length; 3.4  $\mu$  to 4.2  $\mu$  in breadth; free flagellum 5.3  $\mu$  to 6.9  $\mu$ ; nucleus 3.1  $\mu$  to 4.1  $\mu$  to 2.3  $\mu$ . These measurements differ from those of the English trypanosome except in body length.

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PART (II)MYXOSPORIDIA.

This section deals with the observations made by the writer on some Myxosporidia, parasitic protozoa of fishes. These parasites inhabit organ cavities such as the gall bladder, urinary bladder, uriniferous tubules of the kidney and other tissues. They are characterised by multinucleate trophozoites and by the spores in which at least one polar capsule with a coiled filament is present. It is said that Myxosporidia produce various histopathological changes which hinder the normal functions of the organs concerned, and appear to cause the death of fish. Death is due, not only to these organisms as the primary causes, but also to secondary fungus or bacterial infections assuming an epidemic form.

Some Myxosporidia attach themselves to integument, muscles, fins and gills of freshwater fish and the infection is heavy. When the internal viscera are attacked, the myxosporidian infection is to be discovered only by dissection and microscopical examination.

The writer examined parasites not from the



pathological point of view but for their morphological characters and life cycle. For this purpose fishes were collected from various sources as mentioned in the previous part of this thesis. The method adopted was also the same as noted before. Tissues or organs such as gall bladder, urinary bladder, kidney, liver, spleen, fat-bodies and muscle were fixed in carnoy's fixative for more thorough study in sections. Drawings were made with the camera lucida at a magnification of X2500. The observations are summarized in the following table:-

No.	Species of fish.	No. of fish examined	No. of fish infected	Myxosporidia	Organ infected
1.	<u>Indian fish.</u> <u>Clarias</u> <u>batrachus.</u>	25	20	<u>Myxobolus.</u>	Liver, kidney, gall bladder & gonad.
2.	<u>British fish.</u> <u>Cyprinus carpio</u> <u>(Carp).</u>	30	11	<u>Myxidium</u>	Gall- bladder.
3.	<u>Scardinius</u> <u>erythrophthalmus</u> <u>(Rudd).</u>	6	1	<u>Zschokkella</u>	do
4.	<u>Perca</u> <u>fluviatilis</u> <u>(Perch).</u>	12	1	<u>Henneguya</u>	Gills.
5.	<u>Phoxinus</u> <u>phoxinus</u> <u>(Minnow).</u>	15	1	<u>Chloromyxum.</u>	Gall- bladder.

Description of species

Myxobolus clarii

Parallel studies of descriptions and preparations of Myxobolus clarii of Clarias batrachus of Calcutta (East India) and of Myxobolus from the same species of fish from Hyderabad State (India) prove that the two are the same species but the parasite from Hyderabad is slightly smaller. As the morphological account of this parasite has not been given in much detail by Chakravarti, the previous investigator, it is considered worth describing.

Numerous white nodules or cysts about one to two millimetres in diameter and attached to each other by means of tissue fibres were found studding the surfaces of the various internal organs, ovary and kidney when infected fish were opened up. In one of the fish were found more than fifty small and large cysts scattered over the tissues which were recognizable with the naked eye. The cysts were rounded, oval to ovoid in shape, and opaque white in colour and with a tissue covering over their surface. Light and heavy infections with small, intermediate and large cysts were seen in twenty fish.

A few cysts removed from the above mentioned tissues were teased on slides and examined freshly.



In hanging drop preparations of the contents of one of the tumours (cysts) spores were found corresponding in major characters to those of M. clarii which Chakravarti (1943) found in the same species examined by him. Innumerable mature and many earlier developing stages were observed. Sections of all tissues were cut and stained.

When examined the cyst wall was 4 to 6  $\mu$  in thickness (figs. 337 & 348) and consisted of an epithelial fibrous layer. Inside this the earlier developmental stages are near the periphery and advanced spores in the central portion of the cyst.

#### Morphology:-

The nucleate pansporoblasts are small, round or irregularly shaped. The cytoplasm is granular and, in some, stains lightly (fig. 335) while deeply in others (fig. 335b). In a few forms vacuoles are seen (fig. 335). The large nuclei are situated either near the border of or in the centre of the cell. Chromatin bodies are present on the nuclear membrane. The dimensions of the pansporoblasts are 2.5  $\mu$  by 3.0  $\mu$ .

Binucleate pansporoblasts are irregular in shape and larger in size owing to a short ectoplasmic pseudopodium

(fig. 339). Two large rounded or oval nuclei have chromatoid bodies on the nuclear membrane and the karyosome in the centre. They are up to  $3.25\ \mu$  to  $5.5\ \mu$  long and  $3.0\ \mu$  to  $4.0\ \mu$  broad. Small and large vacuoles are present.

Tetranucleate forms are larger and rather rounded (fig. 342). Their nuclei are large and nearly fill the whole cell. A karyosome is very distinct and chromatoid bodies are on the nuclear membrane. They are  $4.5\ \mu$  to  $5.0\ \mu$  in diameter.

Many young multinucleate forms are seen near the periphery of the cyst. They <sup>may</sup> show eight to twelve nuclei with karyosomes. In older ones twelve to sixteen karyosomatic nuclei are seen.

Four kinds of spores are observed in a cyst smear:-

1. Spores with two medium sized polar capsules at one end. (figs. 357, 358 & 361).
2. Spores with two long polar capsules at one end (figs. 371 & 372).
3. Spores with three small polar capsules at one end (figs. 362 & 374).
4. Spores with four small polar capsules at one end (fig. 377).



These spores generally appear spherical in a front view and slightly pointed anteriorly and rounded at the posterior end in side view (figs. 350 & 351). The shell consists of two equal valves less prominent in front than behind; that is the thickness of the shell valve increases gradually towards the posterior end, but some have equal thickenings. At the anterior end of the spore there is a slightly expanded process where two narrow tubes or foramina open. The sutural ridge is indistinct in stained spores but in a few it is very clear and straight (fig. 351). Some sporocysts (shells) have prominent folds (figs. 361 & 366). In others these folds are not present (figs. 358 & 367). The valvular nuclei usually are situated at the posterior or non-capsular end (figs. 357 & 367). They are usually compact and oval in shape and are usually two in number (figs. 364 & 365) but in some only one is seen while in others there is none.

The sporoplasm possesses the whole extracapsular space of the spore excluding the iodophilous vacuole, and also extends between the polar capsules. (figs. 363 & 370). The two sporoplasmic nuclei are always connected to each other and are equal in size. They have thickenings on the nuclear membrane. Karyosomes have not been seen

in the nuclei of spores. Each spore has a large iodophilous vacuole which varies in size, position and shape. It occupies the greater part of the sporoplasm and in some hardly any sporoplasm can be seen. Vacuoles are either rounded to oval or spherical or subspherical. In elongated oval forms, it is either on the side (fig. 357) or in the centre of the spore (fig. 366).

The polar capsules are well formed and occupy nearly half of the spore. They are typically round based and goblet shaped and equal in size, with their anterior ends drawn out into a narrow short duct. In a very few, one capsule is slightly smaller than the other (fig. 369). Almost all capsules without extruded filaments stain deeply dark blue in Giemsa stain, but in some coiled filaments are distinct and form a number of coils within the capsules. Coils of five to six turns have been observed in fresh and in preserved material. There is usually a small space between them (figs. 357, 358, 363 & 371), but in a few cases they are in contact. A fair number of fully extruded polar filaments with their blunt ends have been seen. They are seven times as long as the spore. Clear narrow ducts in the filament have been observed. To extrude



filaments of spores from nodules preserved in 70% alcohol for eight months, the writer used .5% KOH but they extruded only partly. The capsulogenous nuclei, curved, dot like or crescentic and compact, are situated on or near the bases of the capsules (figs. 357, 360, 363 & 372). In a few they are almost as large as the sporoplasmic nuclei. In some one nucleus may be seen in others they may apparently not be present.

The measurements of the spores are as follows:-

- |     |                                    |                          |
|-----|------------------------------------|--------------------------|
| (1) | Length of spherical spores. ..     | 8.0 $\mu$ to 10.5 $\mu$  |
|     | Breadth of spherical spores. ..    | 7.0 $\mu$ to 9.0 $\mu$   |
|     | Sutural ridge.. .. .               | 0.5 $\mu$                |
|     | Length of the polar capsule. ..    | 3.0 $\mu$ to 5.0 $\mu$   |
|     | Breadth of the polar capsule...    | 1.7 $\mu$ to 2.5 $\mu$   |
|     | Length of iodophilous vacuole. ..  | 2.5 $\mu$ to 4.0 $\mu$   |
|     | Breadth of iodophilous vacuole. .. | 2.4 $\mu$ to 3.5 $\mu$   |
| (2) | Length of the elongated oval       |                          |
|     | spore.. .. .                       | 12.5 $\mu$ to 13.5 $\mu$ |
|     | Breadth of the elongated oval      |                          |
|     | spore.. .. .                       | 8.0 $\mu$                |
|     | Length of the polar capsule. ..    | 7.0 $\mu$ to 9.0 $\mu$   |
|     | Breadth of the polar capsule...    | 2.0 $\mu$ to 3.0 $\mu$   |
| (3) | Length of spore with 3 polar       |                          |
|     | capsule. .. .. .                   | 8.5 $\mu$ to 10.0 $\mu$  |
|     | Breadth of spore with 3 polar      |                          |
|     | capsule. .. .. .                   | 7.0 $\mu$ to 9.0 $\mu$   |
|     | Length of polar capsules... ..     | 2.5 $\mu$ to 3.0 $\mu$   |
|     | Breadth of polar capsules.. ..     | 1.5 $\mu$ to 2.0 $\mu$   |
| (4) | Length of spore with 4 polar       |                          |
|     | capsules... .. .                   | 10.0 $\mu$ to 11.0 $\mu$ |
|     | Breadth of spore with 4 polar      |                          |
|     | capsules... .. .                   | 9.0 $\mu$ to 10.0 $\mu$  |

Systematic position:

Regarding its affinities, Myxobolus clarii has spores resembling the spores of Myxobolus orbiculatus (Kudo 1920) in general morphological characters but they differ from the latter in vegetative forms, in not having folds or markings on the shell of the spore and in dimensions.

Ray (1933a) recorded for the first time this myxosporidium from the ovary and the liver of Clarias batrachus. Chakravarti (1943) has described M. clarii from the fish in question. He found this organism on various organs in the form of cysts. He observed only mature spores in the gall bladder as well as in the cysts distributed in liver, fat bodies and gonads. The dimensions he gave differ somewhat from the spores the writer found. The writer has found all the vegetative forms in the cyst and as well as spores with two to four polar capsules. This is a new record. As far as can be ascertained, the comparison of the spores of Clarias batrachus in the author's material with those described by Chakravarti reveals no difference in the morphology.

Myxidium scardini. n.sp.

This Myxidium has been found in the gall bladder of the Rudd, Scardinius erythrophthalmus. One fish out six showed a light infection of this parasite in the bile.

Morphology:-

Vegetative form: The myxidium, unfortunately, was found in an advanced stage of development. Very few uni-bi and multi-nucleate pansporoblasts were seen. A few stages have been observed in sections of gall-bladder.

The uninucleate pansporoblast is round (figs. 381 & 382) or sub-circular amoeba-like (fig. 386). Its cytoplasm stains rather lightly and has several vacuoles. The nucleus is large with well marked karyosome (fig. 382) and one or two chromatoid thickenings on its nuclear membrane (fig. 382). In size it is about 3.5  $\mu$  by 3.0  $\mu$ .

The binucleate pansporoblast is also sub-circular (fig. 383). The nucleus contains a karyosome and chromatoid thickenings on the membrane. It is 3.5  $\mu$  long and 2.5  $\mu$  broad.

The trinucleate pansporoblast is correspondingly large in size, as compared with the uni and bi-nucleate



forms.

The multinucleate pansporoblast is very large. It apparently produces two sporoblasts which give rise to two spores only, being thus disporous. The cytoplasm is finely granular (fig. 388).

Spore: The spore varies in shape. Generally it is elongated; fusiform in front view (fig. 395) but one valve is arched the other is flat in a side view (figs. 396 & 398). The shell valve is slightly asymmetrical, without any longitudinal striations. The extremities are somewhat bluntly pointed with openings for polar filament extrusion (figs. 393 & 395). The sutural line is delicate and situated crossways (figs. 392 & 396) although in some it is straight (figs. 397 & 398). The sporoplasm is finely granular and contains two nuclei in the centre of the spore. They are always found together arranged either one behind the other or side by side (figs. 392, 395 & 399). In one case three nuclei were present which is thought to be abnormal (fig. 394). All nuclei have distinct karyosomes and chromatoid thickenings on the nuclear membrane.

The polar capsules are situated one at each end of the spore. They are broadly pyriform (figs.

392 & 395) or sub-spherical (figs. 396 & 398) in shape. They are  $4.0\ \mu$  long and  $3.0\ \mu$  broad. The capsulogenous nuclei are elongated slender and crescentic (figs. 394 to 396) but some may be thick and large in size (fig. 391).

The valvular nuclei in most of the spores are not present but in some they are small elongated masses. A coiled polar filament is seen in lightly stained spores which is arranged in a screw-thread like manner (fig. 389). They extrude under the action of KOH and have the length of  $5.5\ \mu$ .

Systematic position:-

The occurrence of this myxidium in the bile and gall bladder of Scardinius erythrophthalmus is recorded for the first time. Previous work shows that more than thirty species of Myxidium have been described from different species of marine and freshwater fish. Many of them differ from this species in their shape, morphological characters and dimensions. A few which somewhat resemble it will be discussed here.

The sporoplast of this parasite is round or sub-spherical. The spores are elongated and fusiform measuring  $10.5\ \mu$  to  $12.0\ \mu$  long,  $4.0$  to  $5.0\ \mu$  broad;

the polar capsules  $3.5\ \mu$  to  $4.0\ \mu$  by  $2.5\ \mu$  to  $3.25\ \mu$  and polar filament  $40.0\ \mu$  to  $56.0\ \mu$  long. The spore is smaller than the spores of (1) M. lieberkuhni (2) M. histophilum (3) M. pfeifferi and about equal to those of (4) M. macrocapsulare, (5) M. glutinosum and (6) M. phyllium, but the latter three are broader being as  $5.2\ \mu$  to  $8.0\ \mu$  in breadth. (7) M. danilewskyi has spores equal in length but smaller in breadth viz.  $3.0\ \mu$  to  $4\ \mu$ . In addition, it has one nucleus in the sporoplasm. All parasites except Nos. 5 & 7 differ as they have longitudinal striations on their shell whereas M. scardini has no such lines. M. glutinosum has no longitudinal striations but differs in being more pyriform in shape. Its capsules open at the apex of a small conical elevation.

M. phyllium (Davis 1917) resembles the present species in shape of spore, polar capsule and capsulogenous nuclei but differs in having longitudinal striations, great breadth and being polysporous.

On account of these differences this parasite is considered to be a new species, and is named Myxidium scardini n.sp. with characters as detailed herein.

Zschokkella cyprini n.sp.

Heavy infection of Zschokkella spores has been



seen in the bile and bile duct of six carp, Cyprinus carpio, out of fourteen caught from the gravel pit near Shenley. Spores almost all in an advanced stage of development were observed floating in bile. They were colourless and transparent. A drop of .5% NaOH was used to extrude the polar filaments which shot out from their capsules forcibly. Sections of liver and gall-bladder were cut and stained with Giemsa differentiated by colophonium acetone. Some earlier developmental stages have been observed in the hepatic bile duct.

Uninucleate pansporoblasts are oval or circular. Their cytoplasm seems granular, and due to vacuolation it occupies a very small space in between two vacuoles and appears in the form of fibres connected with the nuclear membrane. The karyosomatic nuclei are comparatively large and lie in the centre of the cell. They are 2.5  $\mu$  in diameter (figs. 402 & 403).

Binucleate pansporoblasts are subspherical or irregular in shape with large nuclei. Karyosomes are very distinct and chromatoid bodies are either attached to the nuclear membrane or spread throughout the nucleus. They are 3  $\mu$  to 4.0  $\mu$  long and 2.0  $\mu$  to 2.5  $\mu$  broad (figs. 404 & 405).

The subspherical trinucleate pansporoblasts have large nuclei with distinct karyosome and prominent

nuclear membrane. These large nuclei fill nearly the whole pansporoblast and there is hardly any trace of cytoplasm to be seen. Chromatin bodies are either present inside the nucleus or adherent to the nuclear membrane. They are  $3.0\ \mu$  to  $3.5\ \mu$  long and  $2.5\ \mu$  to  $3.0\ \mu$  broad (figs. 407 & 408).

The pansporoblasts are very large and irregular in shape. They are disporous. Their cytoplasm is granular. Different types of nuclei are present in the pansporoblasts, namely:-

1. Large nuclei with karyosome.
2. Nuclei with beaded nuclear membrane as predecessors of sporoplasmic nuclei.
3. Elongate oval nuclei in the valve.
4. Smaller rounded nuclei representing precapsulogenous nuclei.

The polar capsules when differentiated appear as refractile rounded area in the living specimen. Sporoplasts measure  $11.0\ \mu$  to  $15.0\ \mu$  by  $7.0\ \mu$  to  $10.0\ \mu$ .

In an older sporoblast (fig. 417) the polar capsules of two spores are prominent and more developed and segregation into two spores can be seen. Large pansporoblasts (figs. 411 to 416) may contain several sporoblasts in various stages of differentiation at the same time. It is said when sporoblasts are well

developed that they may separate from the pansporoblasts and become free or they may form two spored bodies while within the mother cell.

The spores are drum-like with rounded and convex ends. The vacuolar margin is rather thick and equal, but valvular nuclei have not been seen. The sutural line is either curved, S-shaped or straight.

The sporoplasm is finely granular and lies between the polar capsules. The two sporoplasmic nuclei are situated in the middle of the spores, always adhering to each other. They are round or spherical with distinct karyosomes. In some, well marked chromatin granules on the nuclear membrane are present.

The polar capsules one on each end, are round and spherical. In some a very short duct is seen. The large capsulo-nuclei are very distinct, they are attached to the capsule and both may be on the same side or one on each side of the spore. In shape they are oval or crescentic or bean-like. Capsules are 3.5 u to 4.0 u in diameter. The fully extruded polar filaments are 40.0 u to 55.0 u long.

#### Systematic position:-

This species is here recorded for the first time in Cyprinus carpio. It was found in four out of



thirty specimens of this species. The genus Zschokkella was created by Auerbach (1910). Z. hildae is his type species, which was found in the urinary bladder of marine fish. Z. hildae is larger than the present species and differs in morphology and dimensions especially in respect of its polar capsules. Z. nova was discovered by Klokacewa (1914) in the gall bladder of Carassius. Its spores are somewhat larger and the polar capsules smaller than those of the organism of the carp. Kudo (1916) found Z. scheilognathi in the gall-bladder of Acheilognathus lanceolatum in Japan. It is also larger in size and differs in appearance from the British species. Z. globulosa was found by Davis (1917) in the urinary bladder of Spheroides maculatus. Its spores are larger, like those mentioned above. Nemeczek (1922) has reported Z. roviggenesis in Scorpaena from Italy. This has four chromosomes in all its stages, and has a much larger spore. The species from the carp is considered to be a new species and is named Zschokkella cyprini n.sp. with characters as set forth herein.

Henneguya psorospermica (Thelohan)  
(figs. 424 to 436)

The Perch, Perca fluviatilis was received from

a school boy who had caught it along with four carp from a pond near St. Albans. All fish were dead on arrival and found to be decomposed when examined post-mortem. On examination the gill contents of the perch contained a species of Henneguya psorospermica. Unfortunately the Henneguya were in an advanced stage of development. Only one earlier stage of the parasite was seen.

Several species of Henneguya have been recorded from Perca fluviatilis:

Thelohan (1895) observed H. psorospermica from the gills of Perch in France. He stated:-

"The spores are elongated; anterior part fusiform and anterior end blunt.

Polar capsules elongated and parallel to each other. Coiled polar filament

visible in fresh condition. Shell

unstriated. Dimensions:- total length

40  $\mu$  in average, largest breadth 7  $\mu$

length of polar capsule 7  $\mu$  to 8  $\mu$ ."

(Cohn) Labbe found H. texta from the gills of German perch. He describes it as follows:-

"Spore narrow with blunt anterior

end. Sporoplasm with 6 horns (no figure to explain this expression).

When kept in water, sporoplasm takes round form and becomes highly refractive. Dimensions:- length 29  $\mu$  to 38  $\mu$ ; length between the tip and the posterior margin of the cavity 15  $\mu$  to 20  $\mu$ ; breadth 9  $\mu$  to 10  $\mu$ ; polar capsule (8-11) 9  $\mu$  by 2  $\mu$ ; length of 'starren faden' 14  $\mu$ ; length of tail 14  $\mu$  to 18  $\mu$ ."

Wegener found H. psorospermica in the branchiae of Esox lucius. The dimensions of the parasite were as follows:- total length 35  $\mu$  to 38  $\mu$ ; breadth 7  $\mu$  to 8  $\mu$ ; length of the spore cavity 15  $\mu$ ; length of tail 15  $\mu$  to 20  $\mu$ ; polar capsules 4.5  $\mu$  to 5.5  $\mu$ .

Observations on the structure and the morphological details of the spores described by the above mentioned observers show that the Henneguya of the British Perch is identical with H. psorospermica discovered by Thelohan.

Chloromyxum phoxini n.sp.

This parasite was found floating in the bile of a very small fish, Minnow (Phoxinus phoxinus) caught in Lake Windermere. The fish when measured was only 3" in total length. The gall-bladder was very small and only one slide could be prepared from the bile. After having



obtained the bile on a slide, the gall bladder was fixed with a small piece of liver in Carnoy's fluid. Examination of sections stained with Giemsa and Heidenhain's iron haematoxylin (watery) showed the presence of various developmental stages.

The smear preparation showed that only the developed forms of this parasite float freely in the bile while earlier stages remain attached to the epithelium of the gall bladder.

#### Vegetative forms:

The uninucleate pansporoblast is a small round body with a noticeable ectoplasmic pseudopodium (fig. 437). The cytoplasm is of alveolar structure and granular. The nucleus is situated in the middle of the sporoblast. It is  $4.5\ \mu$  in length and  $3.0\ \mu$  in breadth. The trinucleate pansporoblast has an irregularly shaped outline. It measures  $6.5\ \mu$  long and  $5.5\ \mu$  broad. The cytoplasm is granular with three nuclei.

#### Spores:

The spores are spherical in front view (figs. 443, 444 & 445) and oval in side view (figs. 446, 447, 451 & 454). The shell of the spore is comparatively

thick and consists of two equal valves. Each valve has marked straight, slightly curved or zig-zag formed striations, 7 to 16 in number which seem to run towards the centre of the spore. (figs. 440 & 442). 22 to 24 clear markings can be seen rising from the margin in a front view of the spore which appear in a cog-wheel like form (figs. 443 to 445). Valvular nuclei are not present.

The sporoplasm is finely granular. A few are seen vacuolated (fig. 444). Two round karyosomatic sporoplasmic nuclei of equal size are usually present placed very near to each other or actually adjoining.

Two to four polar capsules (figs. 443 to 446) are situated at the anterior end and occupy much of the spore. Where four capsular spores are present one pair of polar capsules is smaller than the other (fig. 445). Their foramina open at the anterior end of the spores (fig. 446). There is no space between them. The capsulogenous nucleus is crescentic and compact (fig. 446). In some they are apparently absent (figs. 444 & 445).

The polar filaments are very long. Fully extruded filaments are from 40  $\mu$  to 65  $\mu$  long. In fresh preparations coiled filaments are arranged obliquely.

Dimensions of the spores are as follows:-

Length of the body. . . . .	8.0 $\mu$ to 9.5 $\mu$
Breadth of the body. . . . .	7.0 $\mu$ to 8.0 $\mu$
Length of polar capsule. . . . .	2.5 $\mu$ to 4.0 $\mu$
Breadth of the polar capsule. . . . .	1.5 $\mu$ to 2.5 $\mu$
Length of polar filament. . . . .	55.0 $\mu$ to 70 $\mu$

Spores are monosporous (fig. 439) or disporous (fig. 443).

Systematic position:-

So far as is known, twelve species of Chloromyxum have been described from freshwater fish. Most of the species differ from this parasite in morphology, dimensions and shape.

C. dubium was discovered by Auerbach (1809) in the gall bladder of Lota vulgaris. Longitudinal ridges on the shell valve and 4 polar capsules of equal size are present. Chloromyxum phoxini lacks these characters and it also differs in dimensions.

Awerinzew (1913) found C. magnum in the gall bladder of Acanthias blainvillei. The spores are comparatively elongated or spherical with 4 polar capsules at the narrow anterior end. They are much longer being 40  $\mu$  to 48  $\mu$  in length, 30  $\mu$  to 38  $\mu$  in breadth and 12  $\mu$  to 15  $\mu$  polar capsules. C. phoxini



differs in the above mentioned characters and dimensions.

Kudo (1916) described C. misgurni in the gall bladder of Misgurn<sup>n</sup> anguillicaudatus. Its spores are spherical but have fine longitudinal striations running parallel to the sutural line. Polar capsules are at the anterior end. The species which the writer found differs in not having longitudinal striations and also in dimensions. Polar filaments of C. misgurni are 28  $\mu$  to 35  $\mu$  long whereas those in C. phoxini are 40  $\mu$  to 65  $\mu$  long.

C. trijugum is found in the gall bladder of Lepomis megalotis. It resembles the present species in some respects but differs in dimensions.

The record of Chloromyxum phoxini from the Minnow is the first from Great Britain. It appears to differ from previously described species in morphology, habitat and host may be considered a new species and designated Chloromyxum phoxini with characters are herein given.

PART (III).(CILIATA)

This section describes two ciliate organisms, a Cycolochaeta and an Ichthyophthirius which, appear to be species already known. Many ciliates have been investigated from freshwater as well as from marine fishes and from an economic point of view, they may be important as ectoparasitic protozoa since these are frequently the cause of disease among freshwater fish.

Material and Methods:-

Fishes have been collected from a gravel pit near Shenley and from Essex. Fresh material has been examined. Smears were fixed in Schaudinn fixative and stained with Heidenhain's iron haematoxylin. Drawings have been made with a camera lucida with the same magnification mentioned before.

Cycolochaeta domerguei (Wallengren, 1897)

This organism had previously been recorded in Europe by Wallengren (1897) where it primarily affected the Carp. He described the morphology with figures and dimensions.

The writer has found this ciliate in large numbers in the gills and scrapings from the scales from six carps out of thirty. This is a beautiful, symmetrically shaped disc or saucer-like body having a maximum diameter of 45 excluding cilia. It consists of a peristomal surface and basal disc. The organism is held in shape by an exoskeleton of the basal disc, the rest of the body being soft and easily deformed. The basal disc is formed of (1) the three cuticular rings (a) the denticulate, (b) the main cuticular and (c) the outer cuticular, and the locomotor organ with cirri and membranelles. The innermost denticulate ring is of 28 to 32 denticles, each of which is equipped with a ring formed of 28 to 32 brush like hooks on the outer side and of the same number of spines on the inner side. The main cuticular ring is marked by radial rays and the outer one with cilia and cirri.

This parasite was first described by Jackson (1875) and then by Wallengren (1897). The latter investigator added considerably to the knowledge of the morphology of several species. Lastly MacLennan (1939) gave a very complete description of this organism.

The form seen by the writer corresponded in dimensions and morphology with Cyclochaeta domerguei,



except that its denticulate rings are wider, the hooks brush like and the number of hooks and spines greater than in MacLennan's description.

Besides this form, there has been found another form in which the denticulate ring is shorter with typically arranged hooks and sharp curved spines. Outside near this ring there is another denticulate ring consisting of 30 to 32 denticles each of which has a fine hook but without spine. This has been recorded by Wallengren (1897).

Ichthyophtherius multifiliis (Fouquet)

This ciliate has been found by the writer in the muscle of two bullheads (Cottus gobio) obtained from Lake Windermere. There were two swellings on the skin near the ventral aspect of the fish. When the skin was removed four or five egg-shaped white nodules having the length of 2 m.m. were found. One nodule was put on a slide with a drop of saline and teased with a fine needle. Innumerable small daughter ciliates were found moving round with the help of cilia when examined under a high power of the microscope. Many cysts were seen containing such daughter ciliates. The adult form of I. multifiliis has not been seen. It is said such adult

forms when fixed to the skin gradually become embedded in the epidermis where multiplication takes place. Finally a white pustule is formed which ruptures and the enclosed ciliates are discharged into the water.



List, with locality of occurrence of Indian, European and British freshwater fishes found parasitized with the seat of infection in each case.

No.	Host.	No. of fish examined.	No. of fish infected	Organ infected	Parasite	Source of host.
1.	<u>Abramis brama</u> (Bream)	31	16	Heart blood.	Trypanosome.	Gravel pit near Colne river, St. Albans.
2.	<u>Anguilla vulgaris</u> (Eel).	70	-	-	-	Norway.
3.	<u>Clarias batrachus</u> .	30	20	Heart blood; liver, kidney gonads and intestine.	Trypanosome; Myxobolus.	Lakes of Hyderabad State, India.
4.	<u>Cobitis barbatula</u> (loach).	6	-	-	-	Fisheries shops.
5.	<sup>t</sup> <u>Cottus gobio</u> (Bullheads)	15	6 2	Heart blood Muscle.	Trypanoplasm Ichthyophtherius.	Lake Windermere.
6.	<u>Cyprinus carpio</u> (Carp).	30	4	Heart blood Gall-bladder.	Trypanosome Trypanoplasm Schokkella.	Gravel pit near Shenley, Trout Fisheries, Essex. Britain.
7.	<u>Esox lucius</u> (Pike).	10	-	-	-	do.
8.	<u>Scardinius erythrophthalmus</u> (Rudd).	6	-	-	-	Fisheries shops.
9.	<u>Mirror carp</u> .	15	7	Heart blood.	Trypanosome	Trout Fisheries. Essex.
10.	<u>Ophicephalus striatus</u>	18	6	do	Trypanosome	Lakes of Hyderabad State, India.
11.	<u>Perca fluviatilis</u> (Perch).	12	2	do	Trypanosome	Lake Windermere and Essex.
12.	<u>Phoxinus phoxinus</u> (Minnow).	15	1	Gall bladder.	Chloromyxum.	Lake Windermere.



No.	Host.	No. of fish examined.	No. of fish infected	Organ infected	Parasite	Source of host.
13.	<u>Rutilus rutilus</u> (Roach).	17	-	-	-	Abbey Lake, St. Albans
14.	<u>Saccobranchus fossilis</u> .	25	4	Heart blood.	Trypanosome.	Lakes of Hyderabad State, India.
15.	<u>Salmo fario</u> (Trout).	28	-	-	-	Lake Windermere.
16.	<u>Salvelinus willoughbii</u> (Char).	29	8	Heart blood.	Trypanoplasm	do
17.	<u>Tinca tinca</u> (Tench)	4	-	do	Trypanoplasm	do



ABSTRACT.

1. Species of Mastigophora, Myxosporidia and Ciliata (known and new) parasitic in freshwater fish are described and illustrated.
2. Trypanosoma striati from Ophicephalus striatus, an Indian fish, is described. It is polymorphic.
3. Culture of T. striati has been obtained in the medium recommended by Shortt. It has been grown on solid medium (plates) for study of developmental stages, nuclear structure and life history.
4. Trypanosoma winchii n.sp., from the mirror carp has been cultivated in the same medium (fluid and solid) mentioned above to compare the Indian and European forms of this trypanosome.
5. If cultures be induced to grow at temperatures lower and higher than 24°C. in the case of the Indian trypanosome and 15°C. for the European trypanosome, they proliferate well but the former die at 34°C. and the latter at 26°C.
6. The first appearance of trypanosomes in primary cultures from blood takes place in between seven

days and one month. When once established, they continue to multiply for an unlimited period after sowing, providing they are sub-cultured every week or fortnight.

7. The trypanosome of Cyprinus carpio when taken with the blood into the crop of a clean Hemiclepsia marginata, starts multiplying after five or six hours. Slender forms are produced 4-5 days later and become very long, attenuated inoculative forms 6-10 days after being ingested. They then pass forward into the proboscis sheath.
8. Trypanoplasma willoughbii n.sp., has been cultivated and maintained at 15°C. temperature in the above mentioned medium. They can grow at a temperature lower than 16°C. but die within a week at 18-20°C. They do not multiply on solid medium. The trypanoplasma from culture can be transmitted into another species of fish by means of inoculation with the syringe.
9. T. saccobranchi var. danilewskyi, T. abramidis. T. percae, T. guernei and T. cyprini have been encountered.
10. Some species of Myxosporidia - Myxobolus clarii, Zschokkella cyprini n.sp., Myxidium scardini n.sp.,



Henneguya psorospermica and Chloromyxum phoxini,  
n.sp., have been investigated from Indian and  
British fish.

11. Two known species of Ciliata - Cyclochaeta and  
Ichthyophtherius have been noted from gills, scales  
and muscles of carp and bullhead.

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Explanation of plate (a)

Photographs of freshwater fishes infected  
with protozoal parasites.

- Fig. 1. Cottus gobio (bullhead) of Lake Windermere, Great Britain with epidermal cyst of Ichthyophtherius multiphiliis, near the anal fin.
- Fig. 2. Clarias batrachus (Maroof) from a river in Hyderabad State, India with heavy infection of blood trypanosomes and myxosporidia.
- Fig. 3. Ophicephalus striatus (Murrel) of the above mentioned State with blood infection of T. striati.

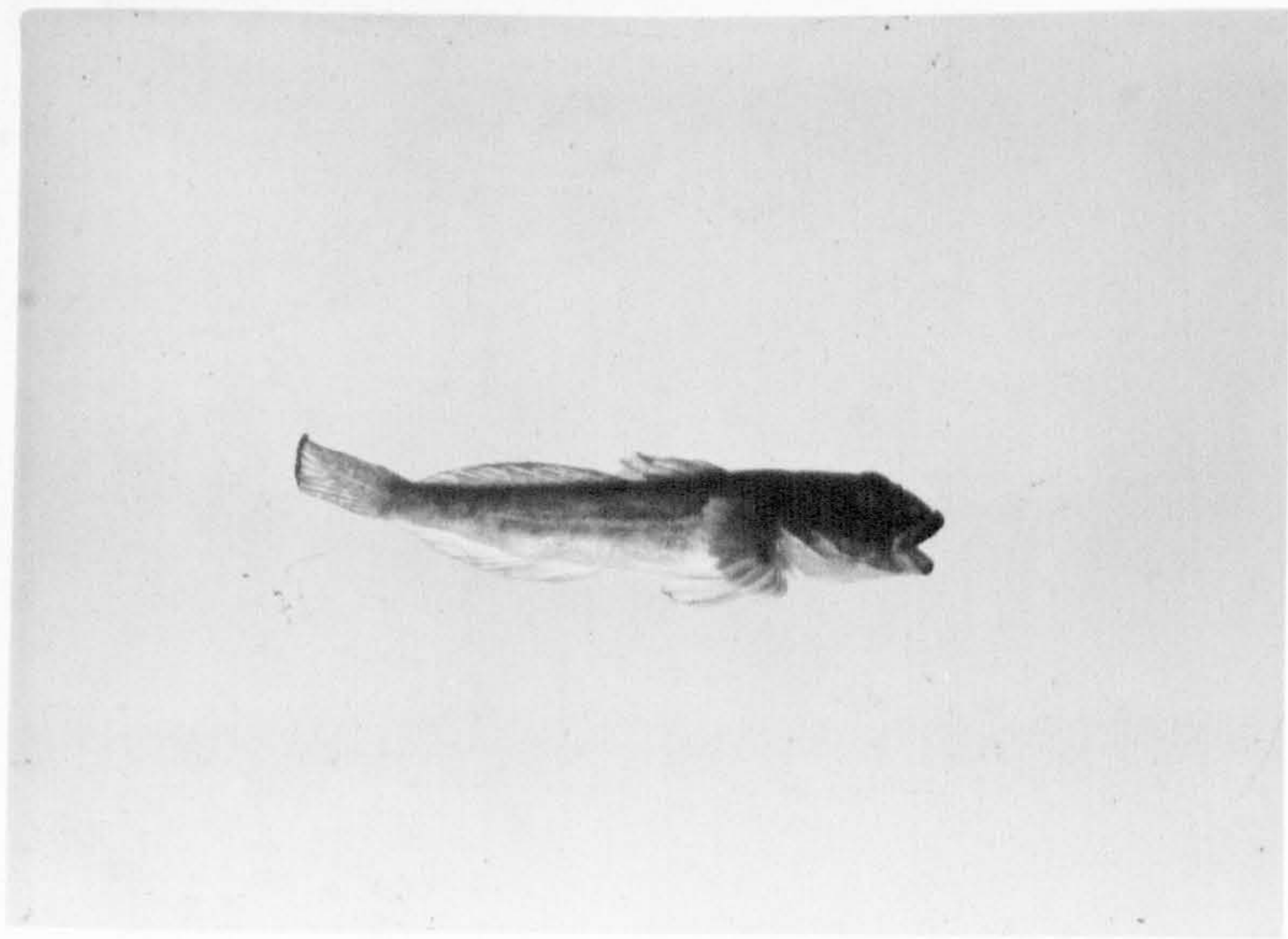


Fig ( 1 )

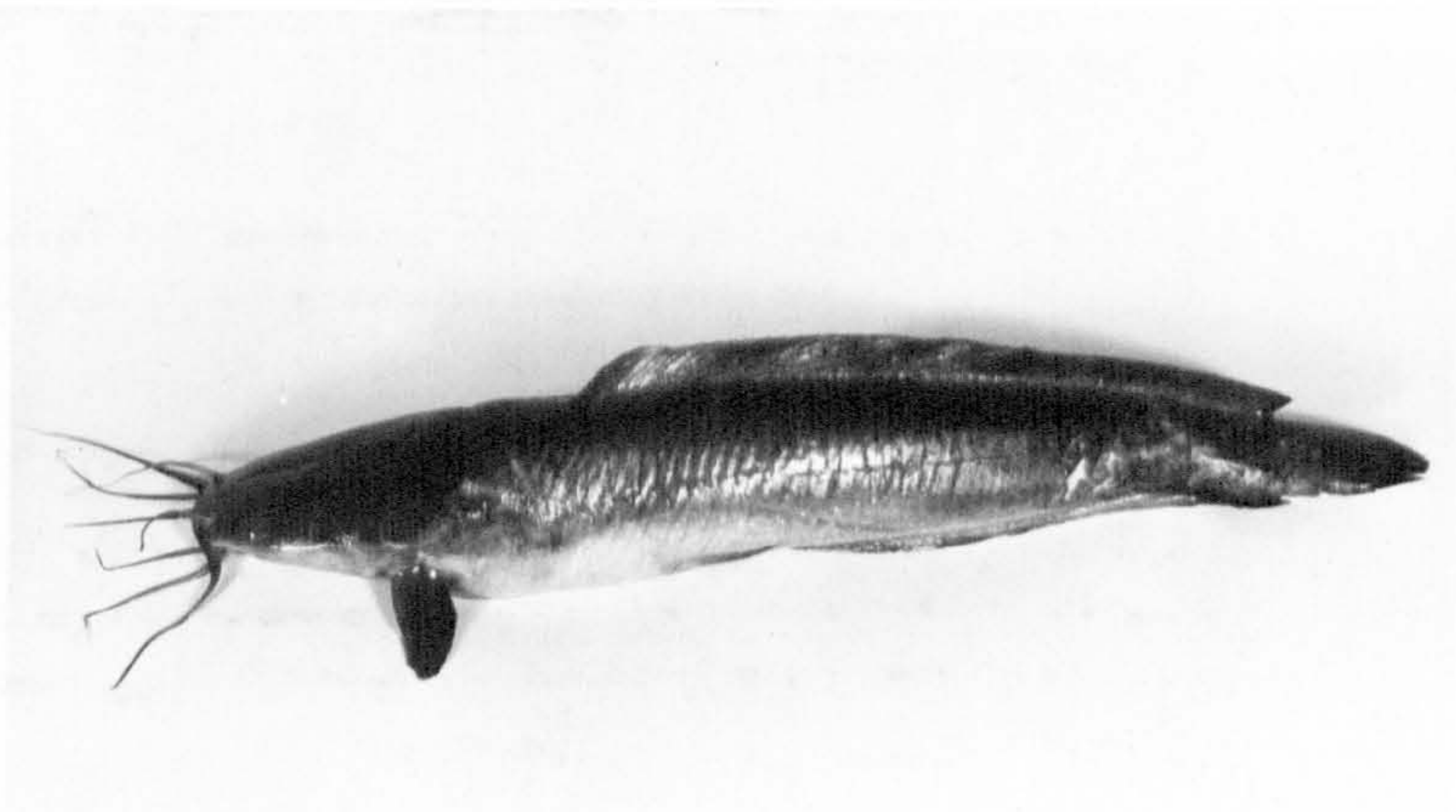


FIG ( 2 )

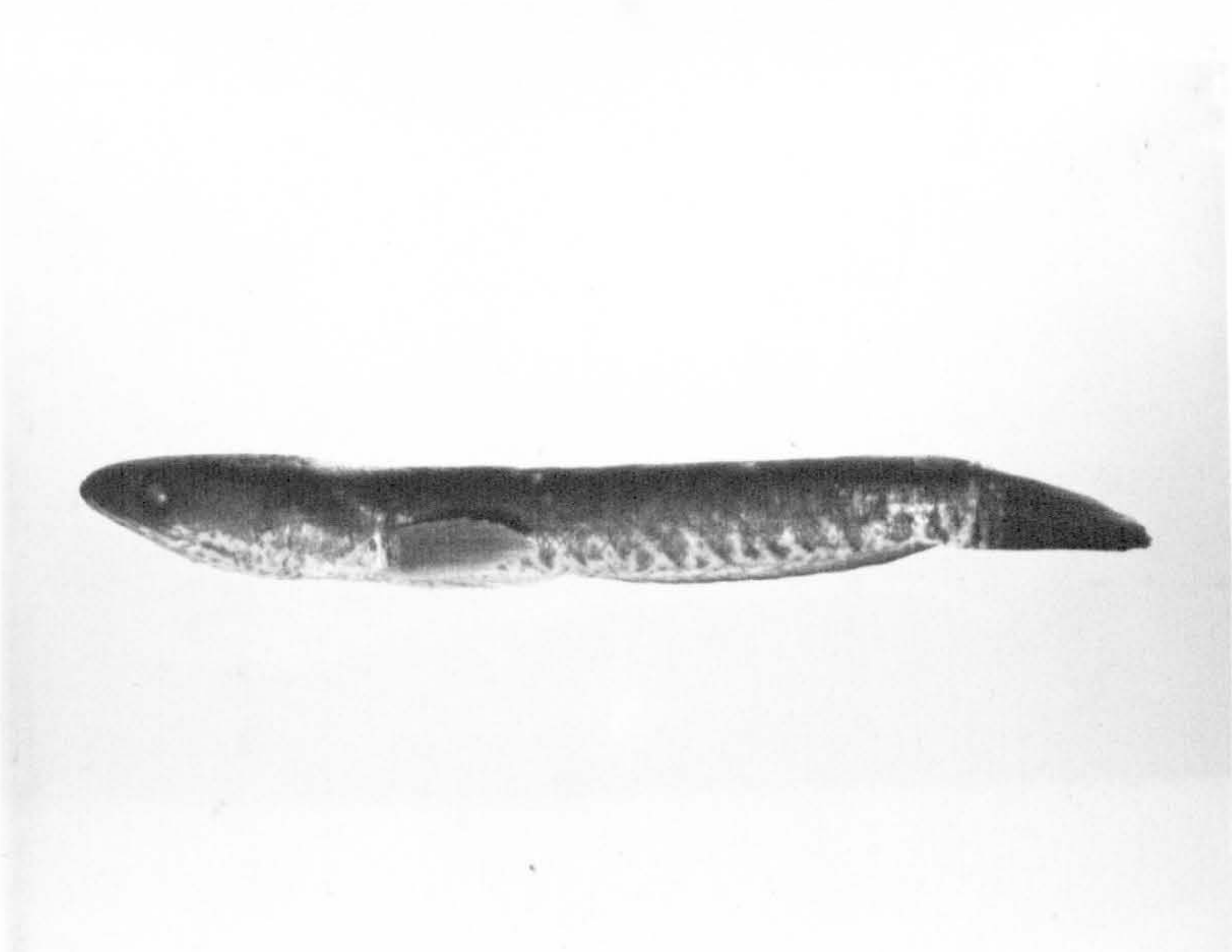




Plate No. I.

Trypanosomes in the blood of fish.

Figs. 1 to 8. Trypanosoma striati of Ophicephalus striatus. All from the same blood but from different slides. Preparations dried off, fixed with methanol and stained with Giemsa.

Fig. 1. Small form.

Fig. 2. Intermediate form.

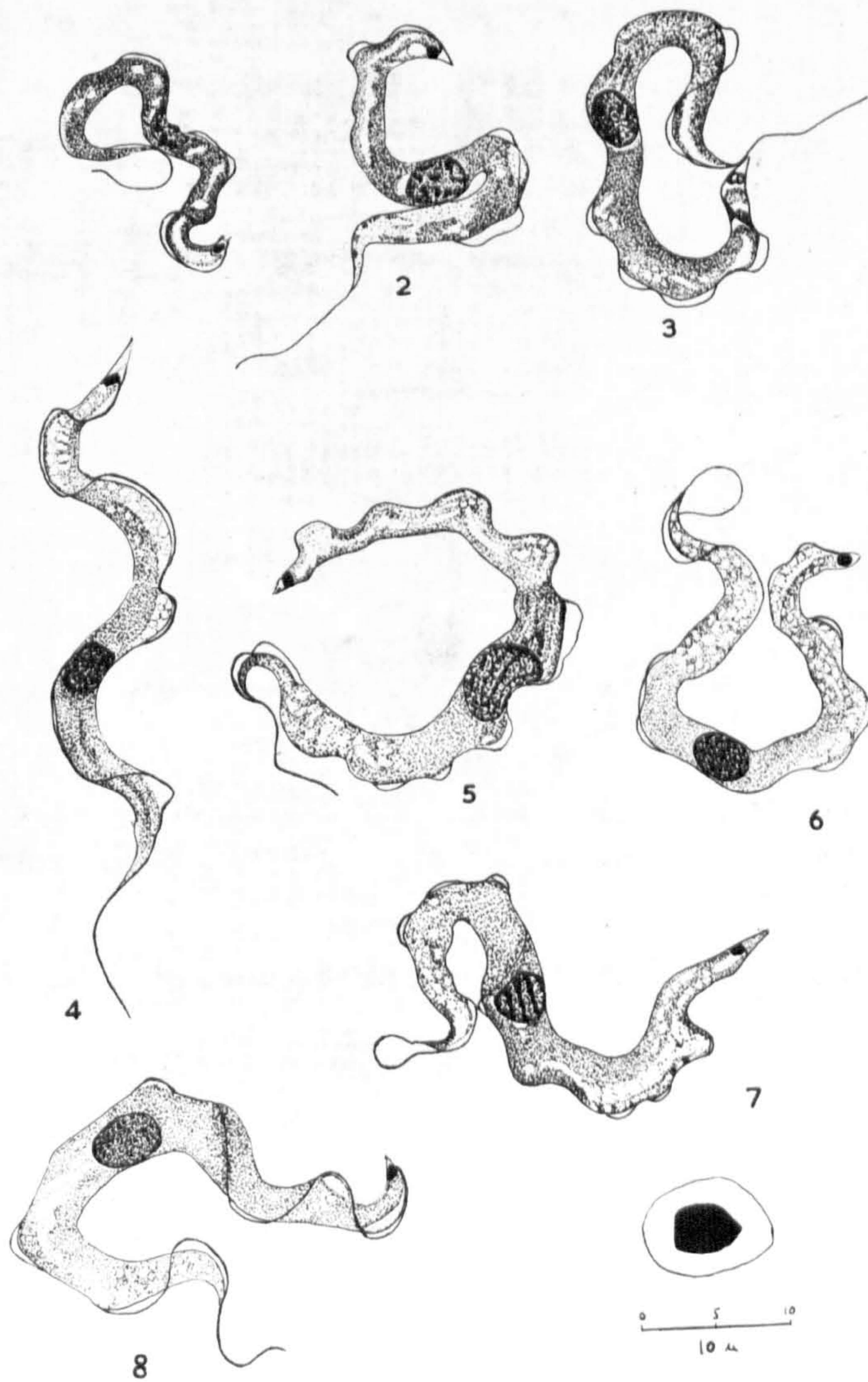
Fig. 3. The same; nucleus oval with nuclear membrane.

Fig. 4. The same; the nucleus in the anterior half of the body.

Figs. 5 & 7. The same; the nuclei are large; chromatoid bodies in it are arranged in rows.

Figs. 6 & 8. The same; the nuclei are oval.

Plate 1

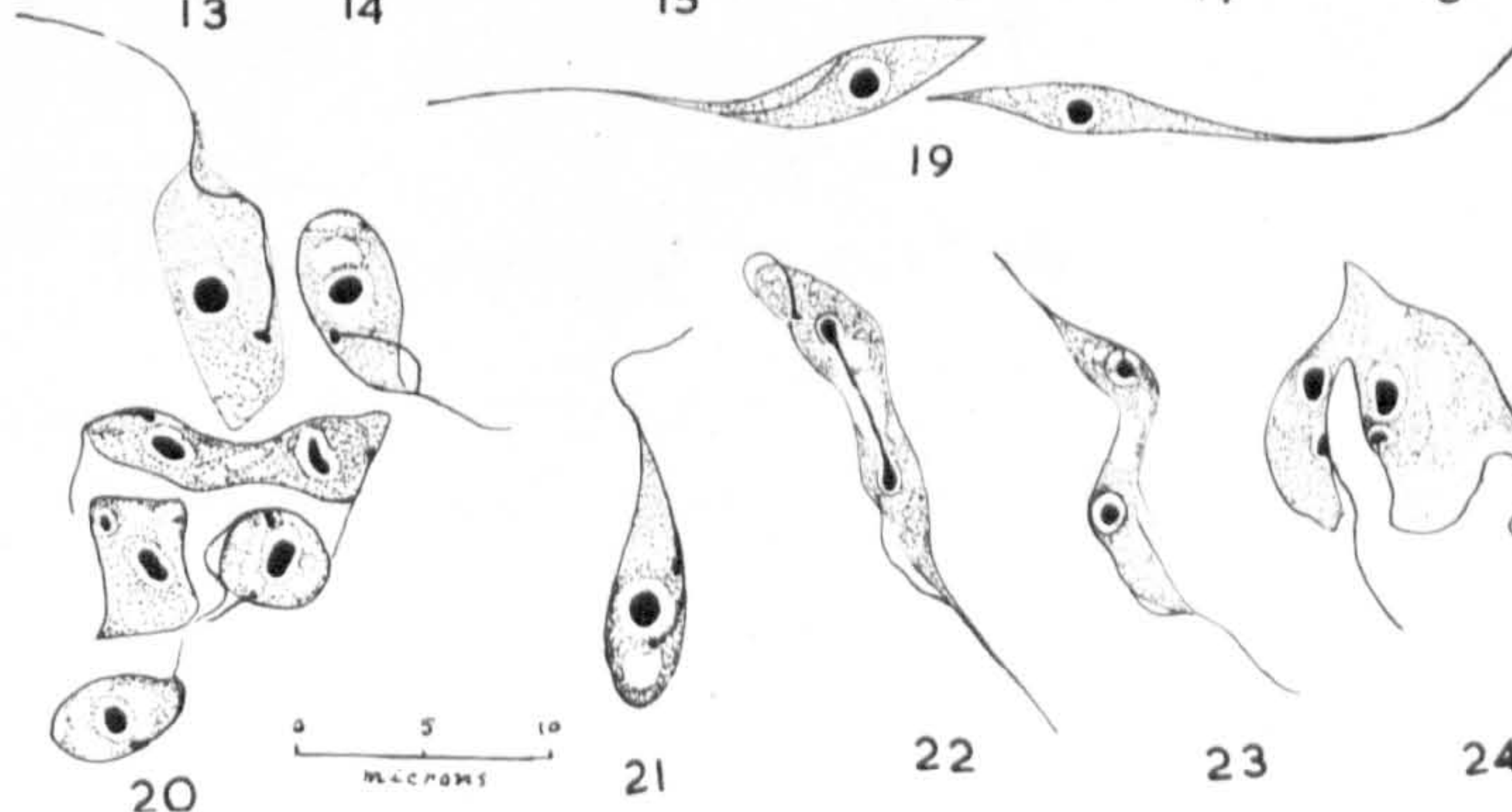
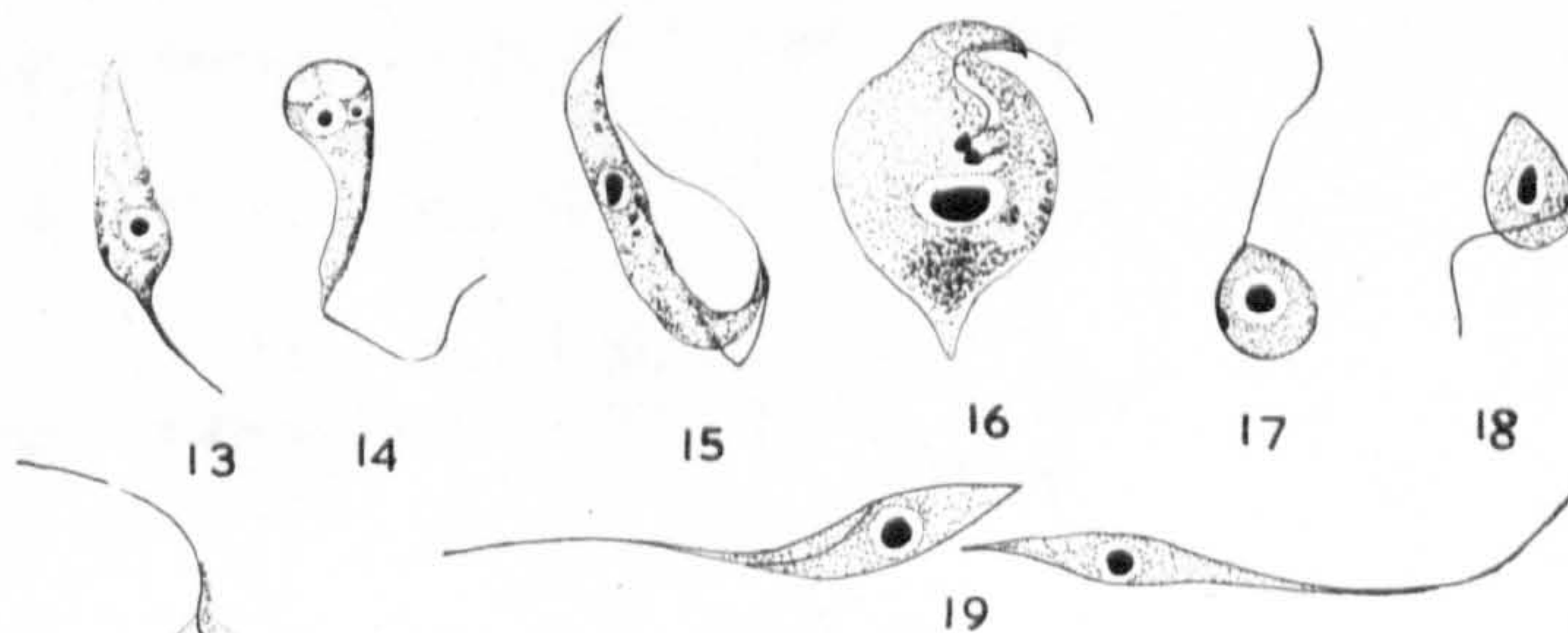
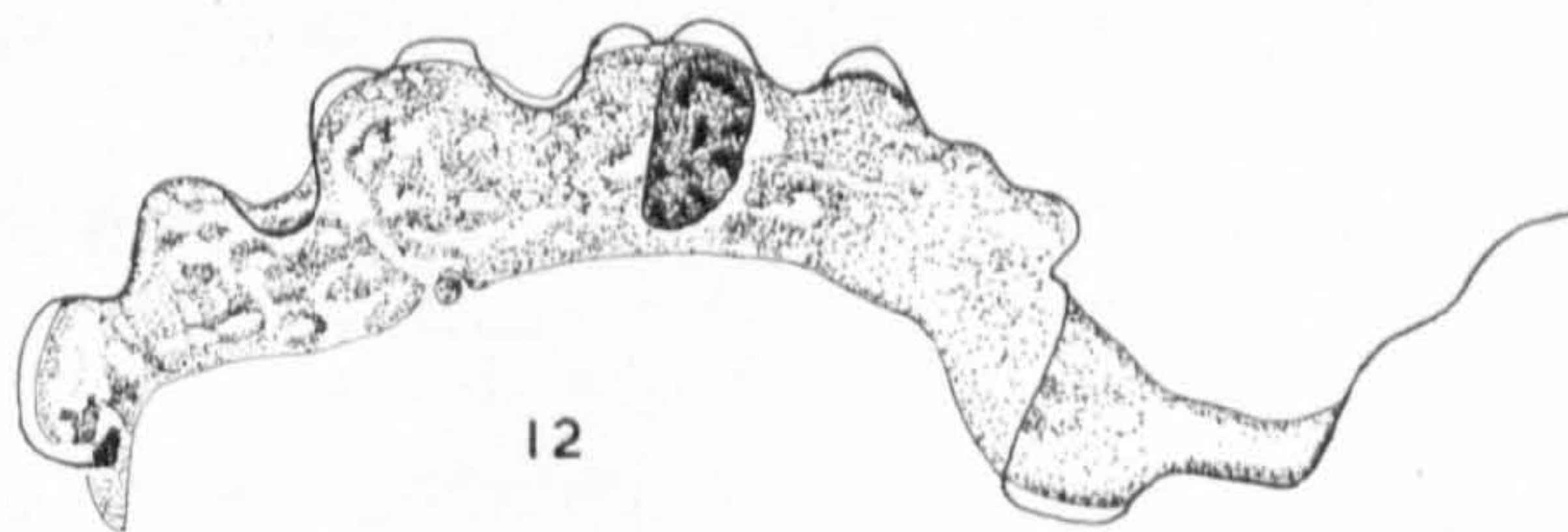
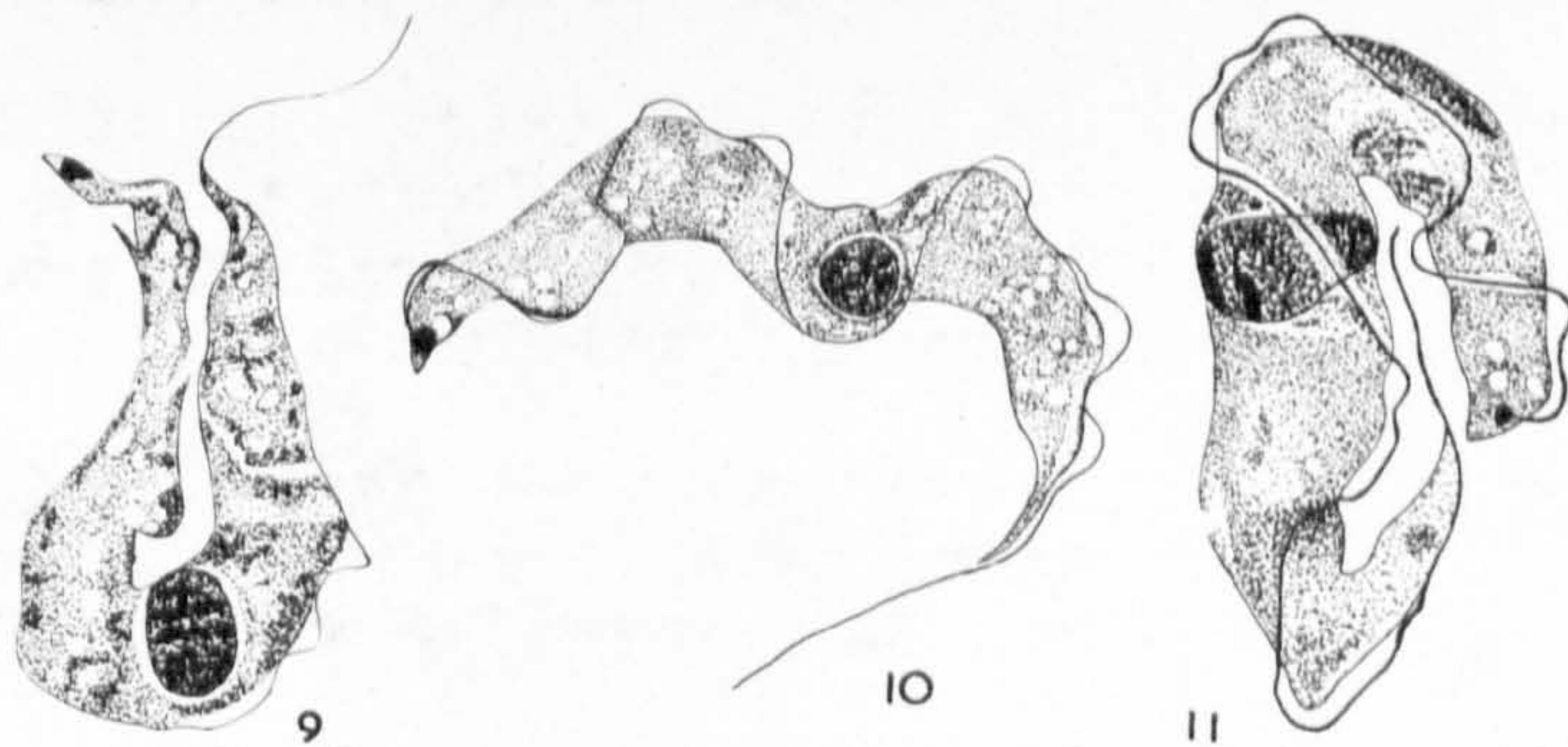




Native and cultural forms of T. striati.

- Figs. 9 to 12. Large forms from the blood of Ophicephalus striatus. Dried off, fixed in methanol and stained with Giemsa. They are large and stumpy forms.
- Figs. 13 to 24. Cultural forms stained with iron haematoxylin.
- Fig. 13. Crithidial form; pyriform; anterior body broad and posterior tapered. Nucleus in the anterior portion with small central dark karyosome.
- Fig. 14. The same; posterior body broad and cobrahood like. Nucleus and parabasal in the broad portion. Free flagellum long.
- Fig. 15. The same; spindle shaped at both ends taper.
- Fig. 16. Globular type; stumpy form. New parabasal body is formed.
- Fig. 17. Globular form; circular nucleus; long flagellum.
- Fig. 18. The same; oval shaped with oval nucleus.
- Fig. 19. The same; lancet like.
- Fig. 20. A small group of crithidial forms; bodies with globular and subglobular and other shapes. Oval nucleus.
- Fig. 21. Crithidial form; club-shaped body; nucleus at the posterior end of the body.
- Fig. 22. Division form; two daughter nuclei connected by cord and appearing dumb-bell shaped.
- Fig. 23. The same; nucleus has divided. The new flagellum reaches the size of the original one.
- Fig. 24. The same; the daughter parabasal body divided before the division of the two daughter parasites.

Plate II



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MICRONS

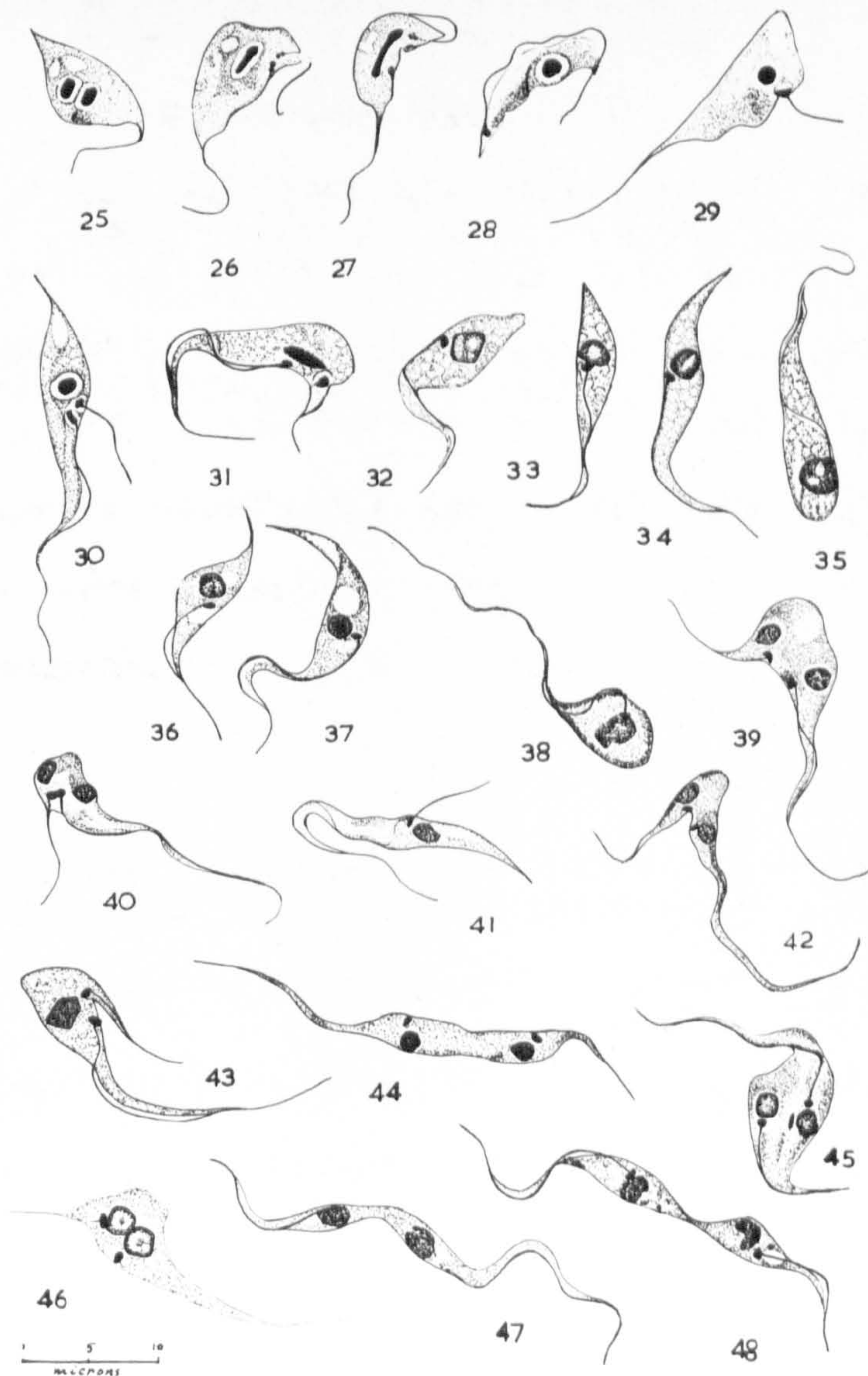


Plate No. III.

Cultural forms of T. striati. Preparations fixed with Schaudinn's fluid and stained with iron haematoxylin.

- Fig. 25. Stumpy form: nucleus has divided before the division of parabasal.
- Fig. 26. Division form; showing a rod-shaped structure, associated with the karyosome.
- Fig. 27. The same; dumb-bell shaped.
- Fig. 28. Trypanosome form with developed undulating membrane. The nucleus is an oval black area surrounded by an achromatic margin.
- Fig. 29. Early form of division; nucleus the same as in fig. 28. New flagellum is seen before the division of the parabasal body.
- Fig. 30. The same; the new parabasal has new flagellum.
- Fig. 31. Dividing form; nucleus ribbon shaped.
- Figs. 32 & 34. Multiplicative types of crithidial forms; Giemsa stain.
- Fig. 33. Fusiform type.
- Fig. 35. The same; club shaped; parabasal and nucleus in the posterior end of the body.
- Figs. 36 & 37. The same; posterior portion fine and pointed.
- Fig. 38. The same; the posterior portion broad.
- Fig. 39. Division form. Complete division of parabasal and nucleus.
- Fig. 40. The same; the nucleus has divided before the division of parabasal.
- Fig. 41. Crithidial form; with the new flagellum.
- Fig. 42. Division form; cytoplasmic division.
- Fig. 43. The same; parabasal bodies separate from each other.
- Fig. 44. The same; contraction of the cytoplasm.
- Fig. 45. The same; division of nucleus and parabasal body.
- Fig. 46 to 48. Stages of division, with contraction of cytoplasm.

Plate III

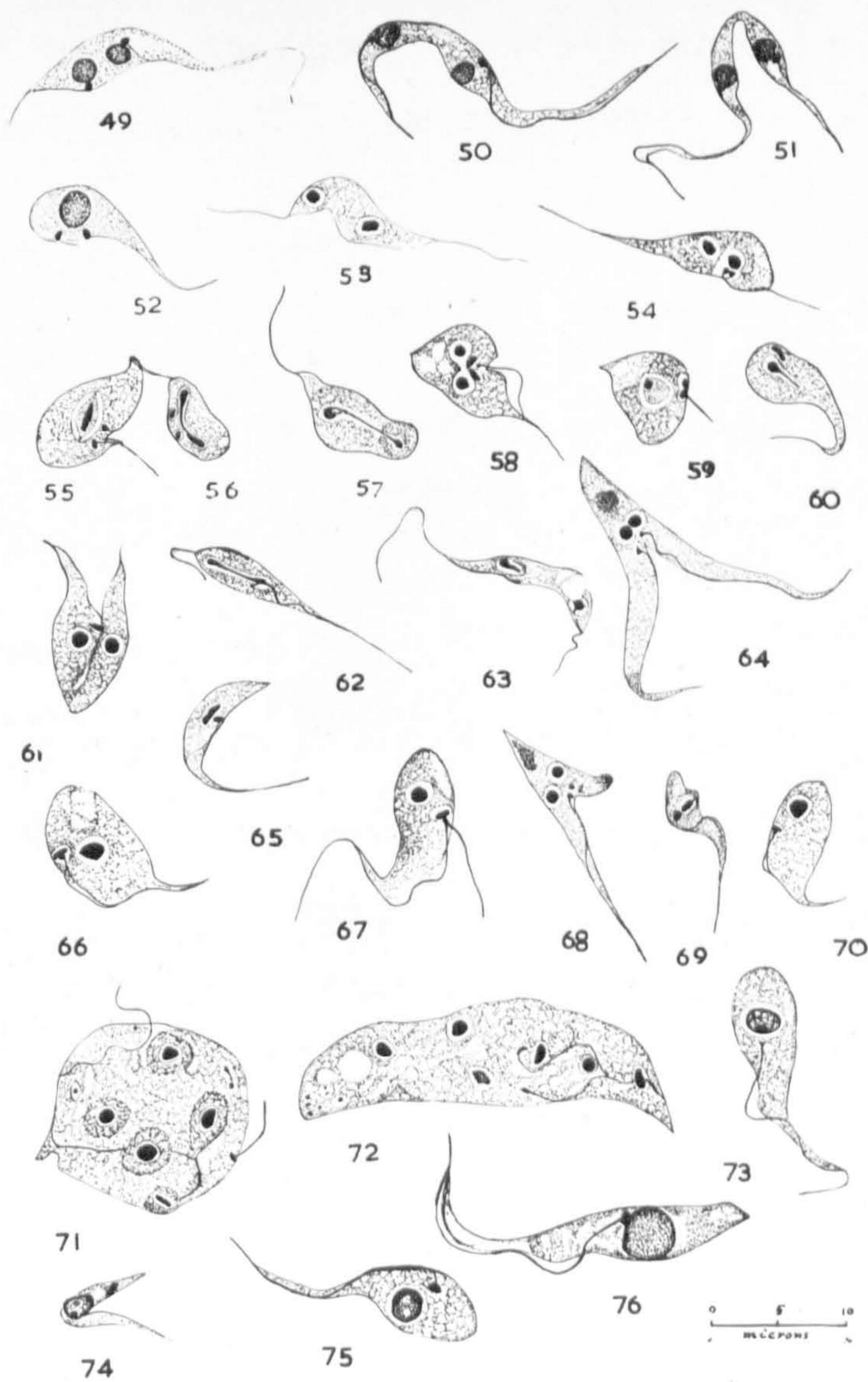




Cultural forms of T. striati Giemsa stain.

- Fig. 49 Dividing forms; distinction between mother  
to 51. and daughter parasites.
- Fig. 52. Crithidial form; newly formed parabasal body.
- Fig. 53. Dividing form; daughter nuclei are formed.
- Fig. 54. The same; division of nucleus before parabasal  
body.
- Fig. 55. Division of cytoplasm. Rhizoplastic area is seen.
- Fig. 56. The same; divided karyosomes linked together  
by a fine thread; new parabasal body.
- Fig. 57. The same; division of the nucleus is unequal.
- Fig. 58. The same; parabasal rod like; a short gap  
between the parabasal and the origin of the  
flagellum.
- Fig. 59. The same; karyosome near the nuclear membrane.
- Fig. 60. Crithidial form; karyosomic mass is connected  
to a rod showing a drum-stick appearance.
- Fig. 61. Dividing form; demarcation line between the  
dividing forms.
- Fig. 62. The same; nucleus is dumb-bell shaped.
- Fig. 63. The same; karyosomic mass like that of fig. 60.
- Figs. 64 The same in longitudinal position.  
& 68.
- Figs. 65 Crithidial form; a ribbon-shaped structure  
& 69. associated with the karyosome.
- Figs. 66 The same; stumpy type; parabasal rod like.  
& 70. Blepharoplast and rhizoplastic area present.
- Fig. 67. Dividing form; new flagellum fairly long before  
the division of parabasal body.
- Fig. 70. Crithidial form; blepharoplast and rhizoplastic  
area present.
- Fig. 71. Large multiple type with four circular nuclei.
- Fig. 72. The same with six nuclei.
- Figs. 73 Crithidial forms.  
to 76.
- Fig. 76. Crithidial form with oval parabasal body.

Plate IV





Cultural forms of T. striati. (haematoxylin).

- Fig. 77. Globular form with one fold of undulating membrane.
- Fig. 78. Body pyriform with small nucleus; karyosome is connected to nuclear membrane by means of radiating fibres.
- Fig. 79. Karyosome is connected just like that of Fig. 78.
- Fig. 80. Tadpole like type; streak is seen.
- Figs. 81 & 82. Small individuals with the parabasal bodies at the posterior end of the body; large karyosomes.
- Fig. 83. Crithidial form; karyosomic mass is divided and the masses appear like a dumb bell.
- Fig. 84. The same; dividing karyosome connected with a cord.
- Figs. 85 & 86. The same with karyosomic mass with transitional stages with a short centrodesmose.
- Fig. 87. Two equal karyosomic masses joined together to form a bilobed karyosome.
- Fig. 88. Crithidial form; fully divided daughter nuclei.
- Fig. 89. The same; grains of chromatin are in the space between the nuclear membrane and the karyosome.
- Fig. 90. Fusiform type.
- Fig. 91. Crithidial form; karyosome is seen in Giemsa stained parasite.
- Fig. 92. Fusiform type.
- Fig. 93. Crithidial form; the broad round posterior end of the body with nucleus and parabasal body.
- Fig. 94. Same as fig. 89.
- Fig. 95. Leptomonas-like form (Giemsa).
- Figs. 96 to 98, 102 & 103. Small mature type in Row's medium.
- Figs. 99 to 101. Long slender attenuated trypanosomes with compressed nuclei (at 30°C.).
- Fig. 104. Degenerated form with large and small vacuole.

Plate v

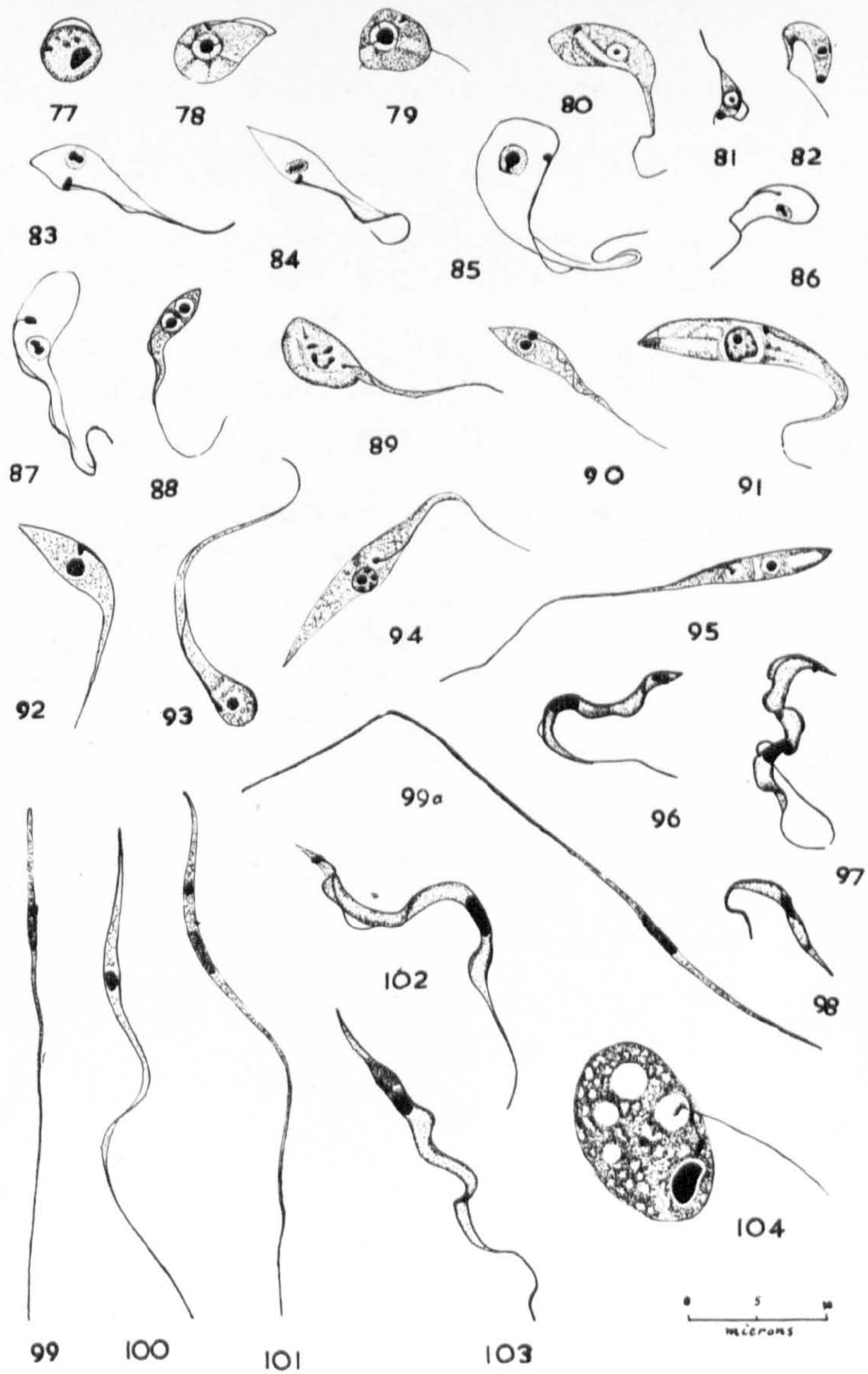




Plate No. (A).

Life-cycle of Trypanosoma striati in the blood of Ophicephalus striatus and in the culture obtained in Shortt's modification of MNN medium.

Figs. 1 to 3. Small, intermediate and large trypanosomes in blood of fish.

Figs. 4 to 20. Developmental stages of trypanosomes in medium mentioned above.

Figs 21 & 22. Mature trypanosomes in Row's medium.

Fig. 4. Crithidial form.

Figs. 5 to 7. The same; division of parabasal and newly growing flagella.

Figs. 8 to 12. Division of nuclei.

Figs. 13 to 20. Separation of newly formed nuclei and the division of cytoplasm.

Figs. 21 to 22. Newly formed small mature trypanosomes.

Plate A.

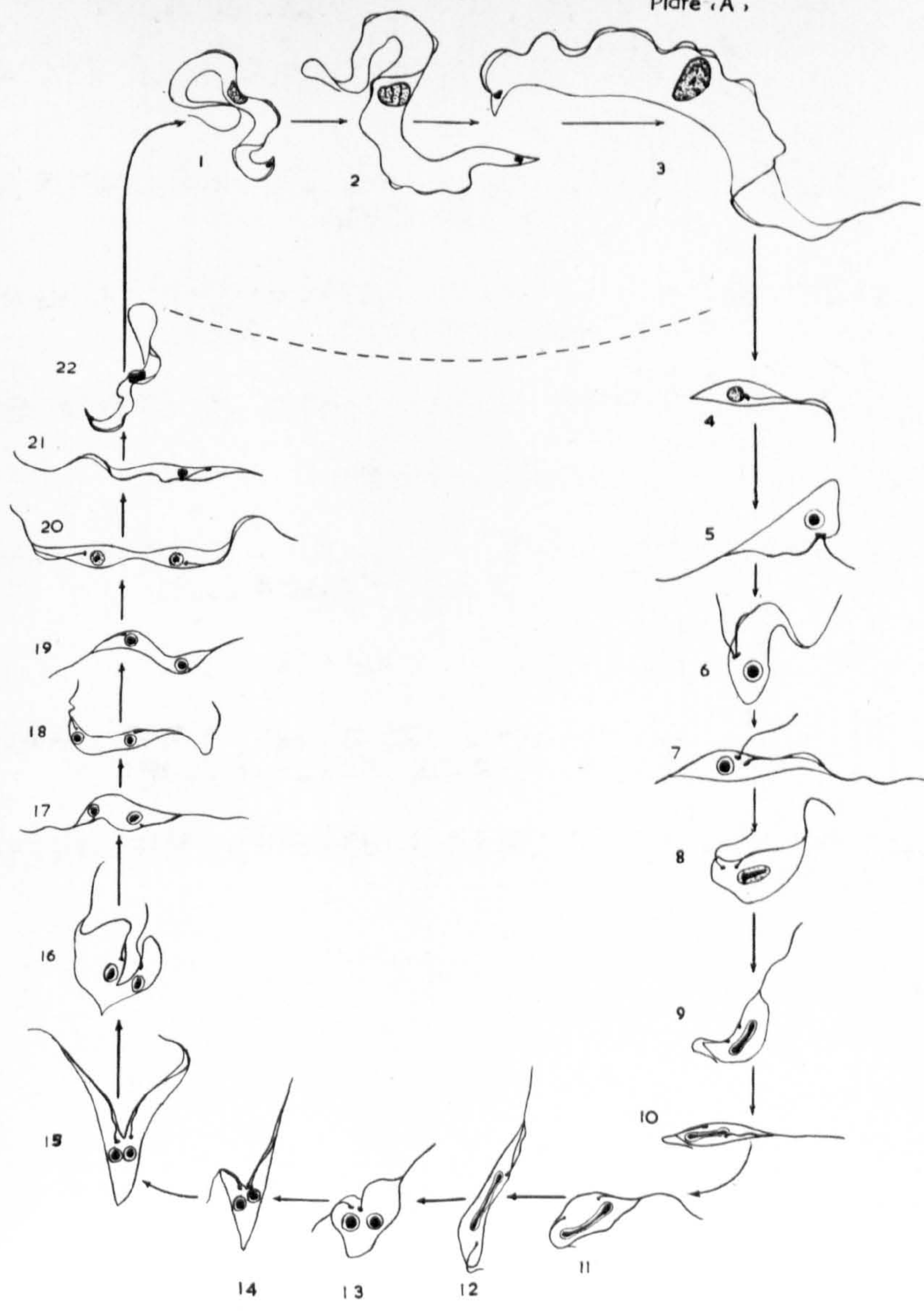




Plate No. VI.

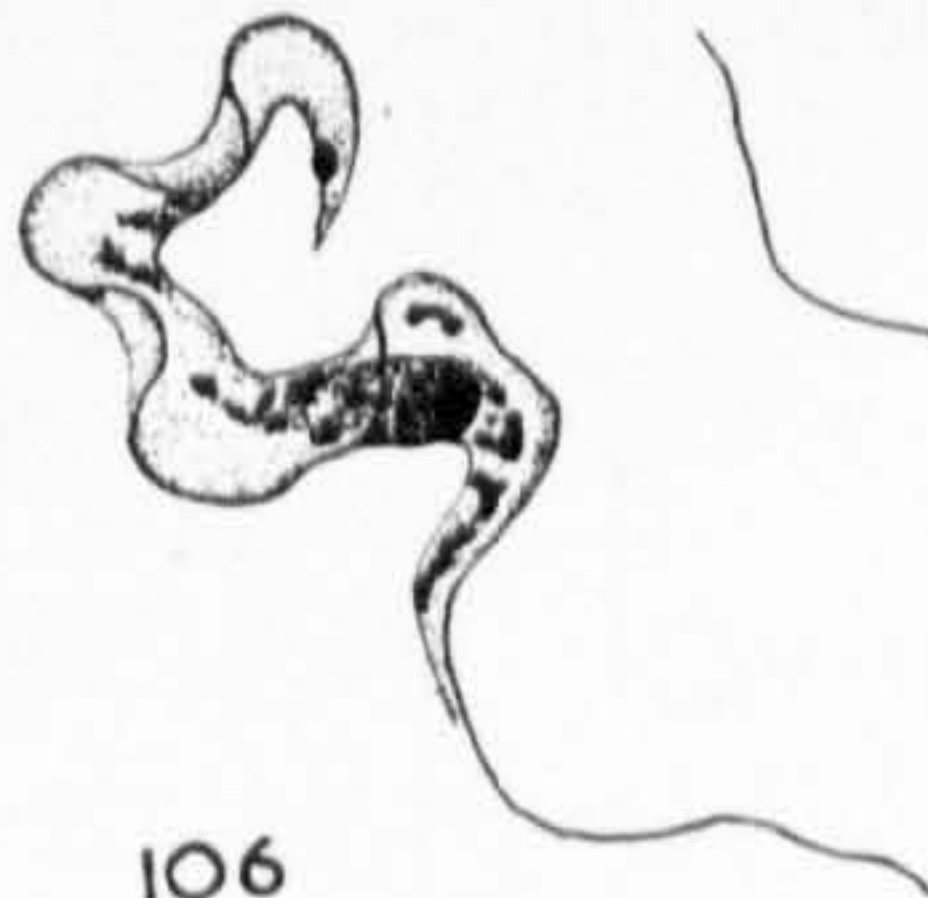
Trypanosoma winchii in the blood of the  
Mirror Carp. All stained with Giemsa stain.

- Fig. 105. Large form with granules arranged in rows.
- Fig. 106. Small form; nucleus near the anterior end  
of the body.
- Fig. 107. Large form.
- Fig. 108. The same.
- Fig. 109. The same; wave like body.
- Fig. 110. The same; many volutin granules.
- Fig. 111. The same.
- Fig. 112. The same; nucleus near the anterior end  
of the body.
- Figs. 113 & 114. The same.
- Fig. 115. The same; karyosome is seen in the nucleus.

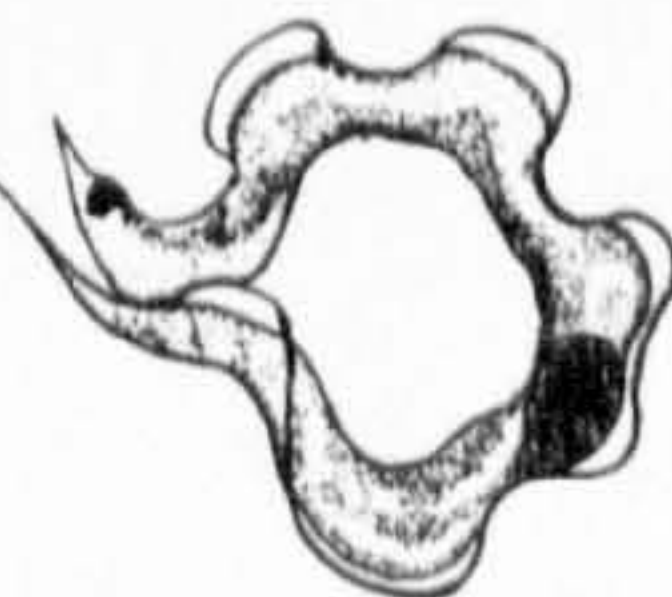
Plate VI



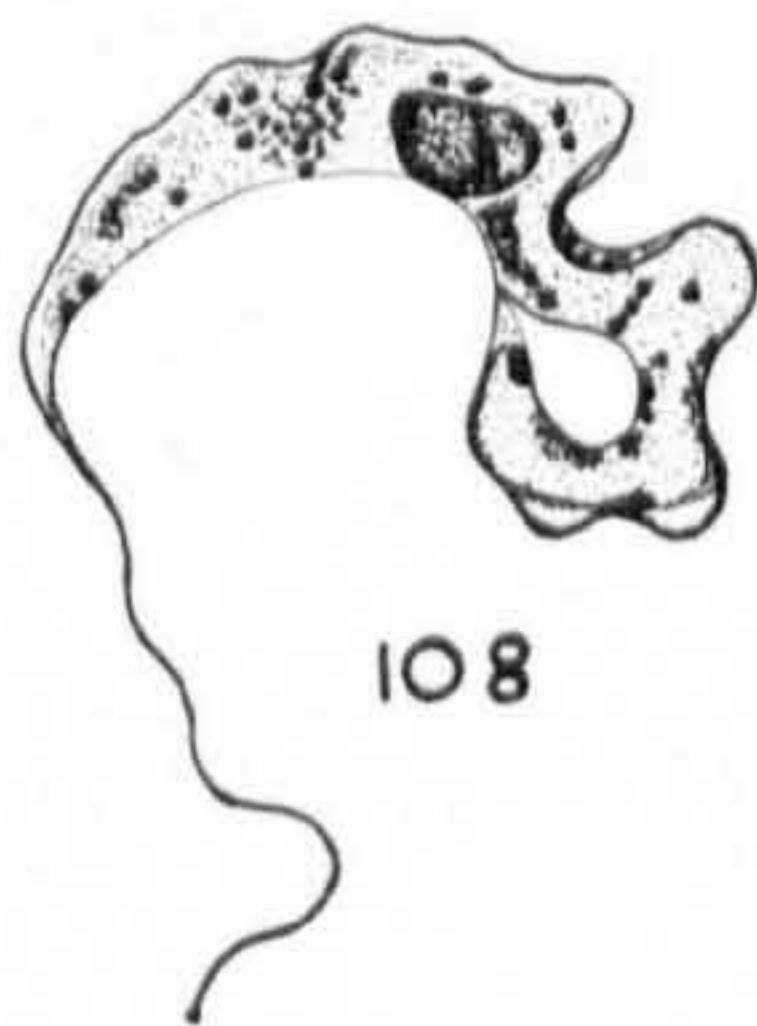
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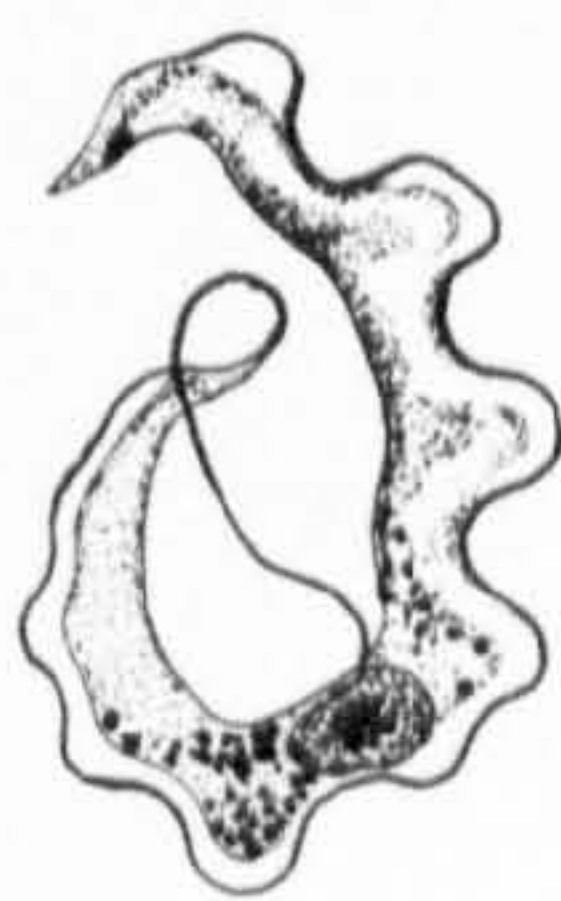
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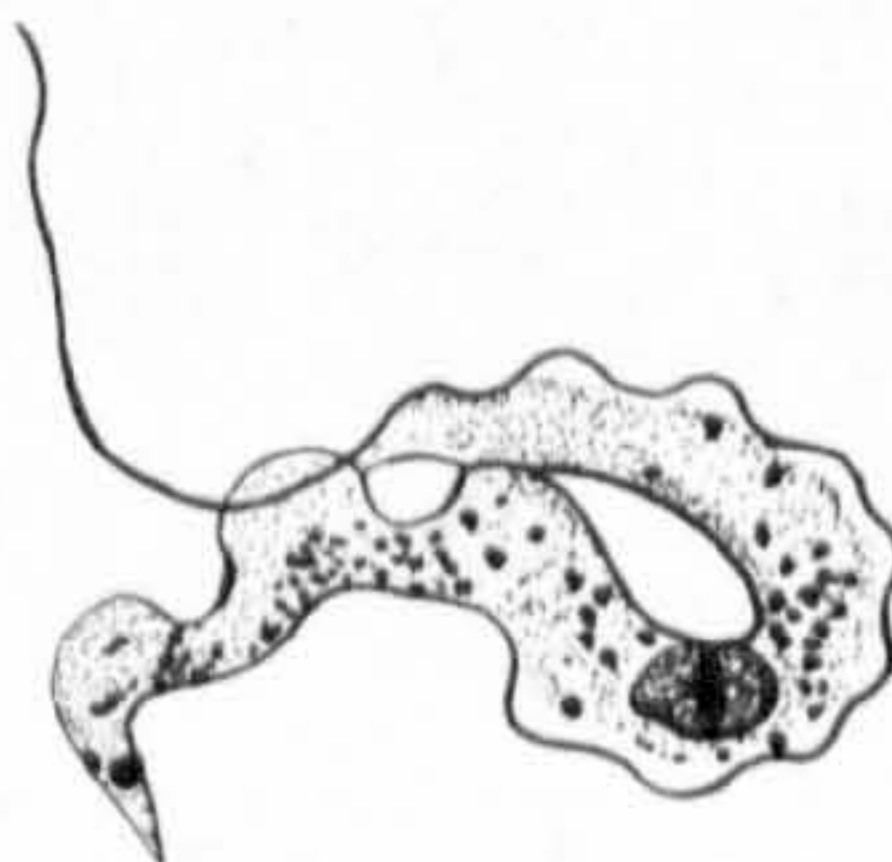
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108



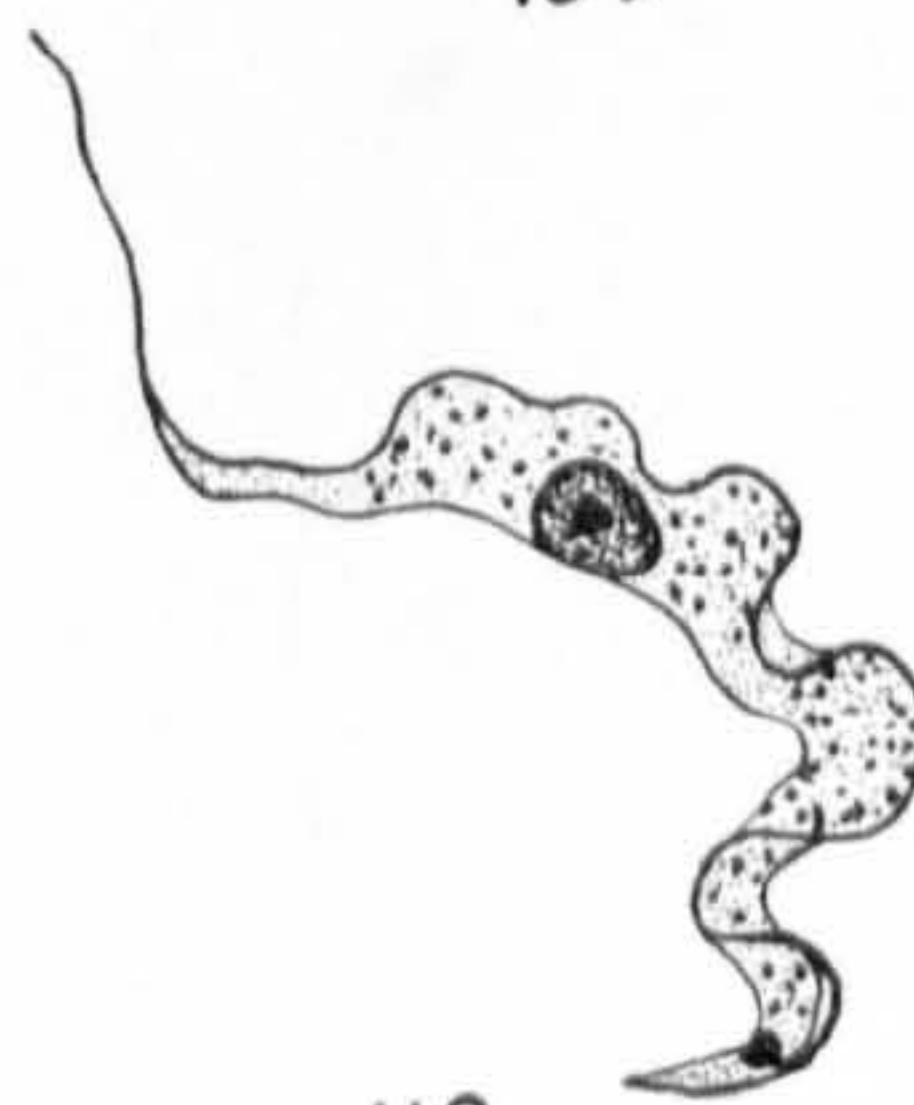
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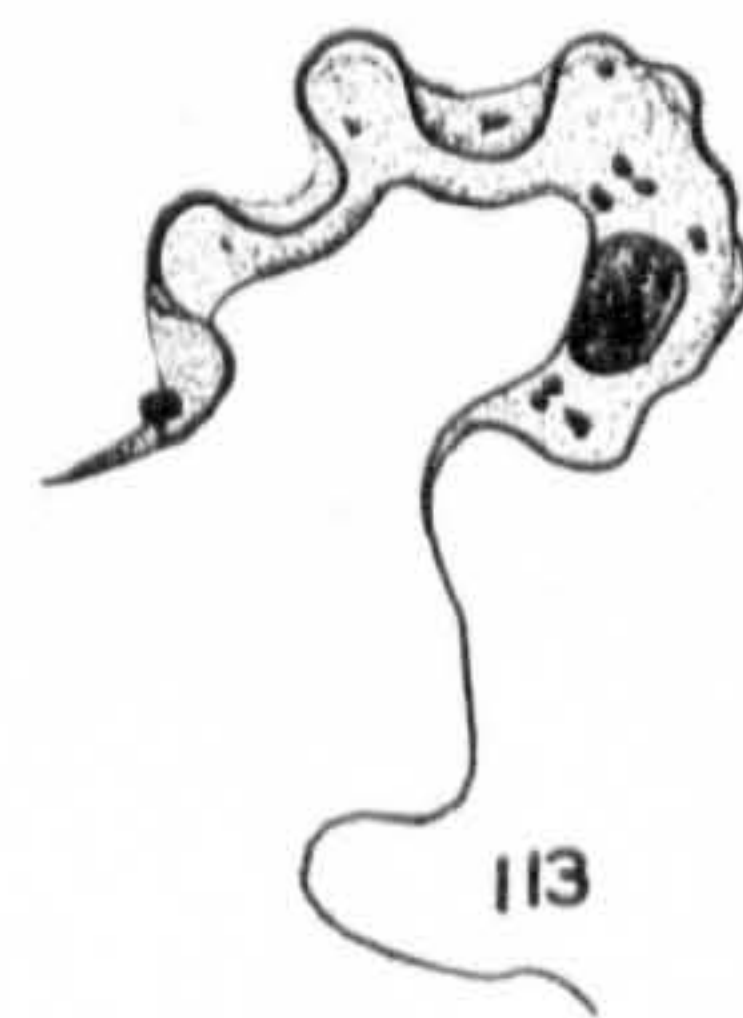
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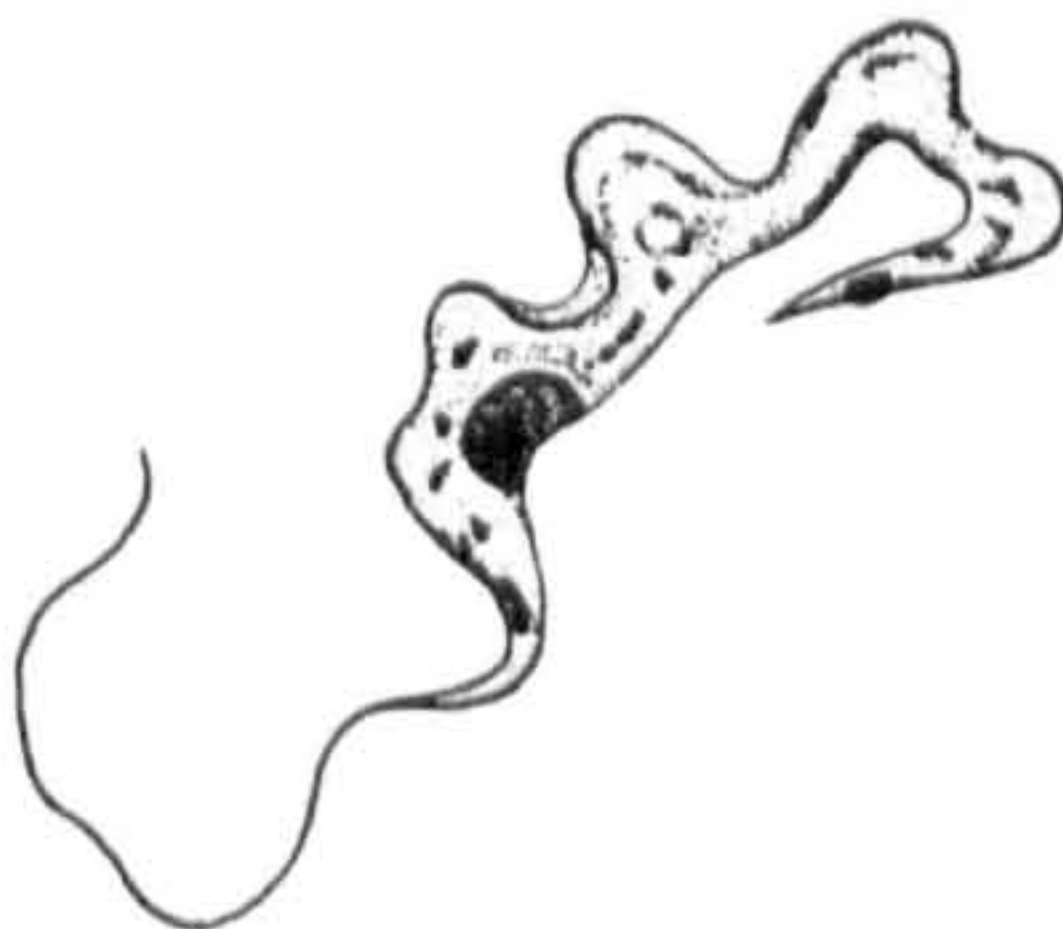
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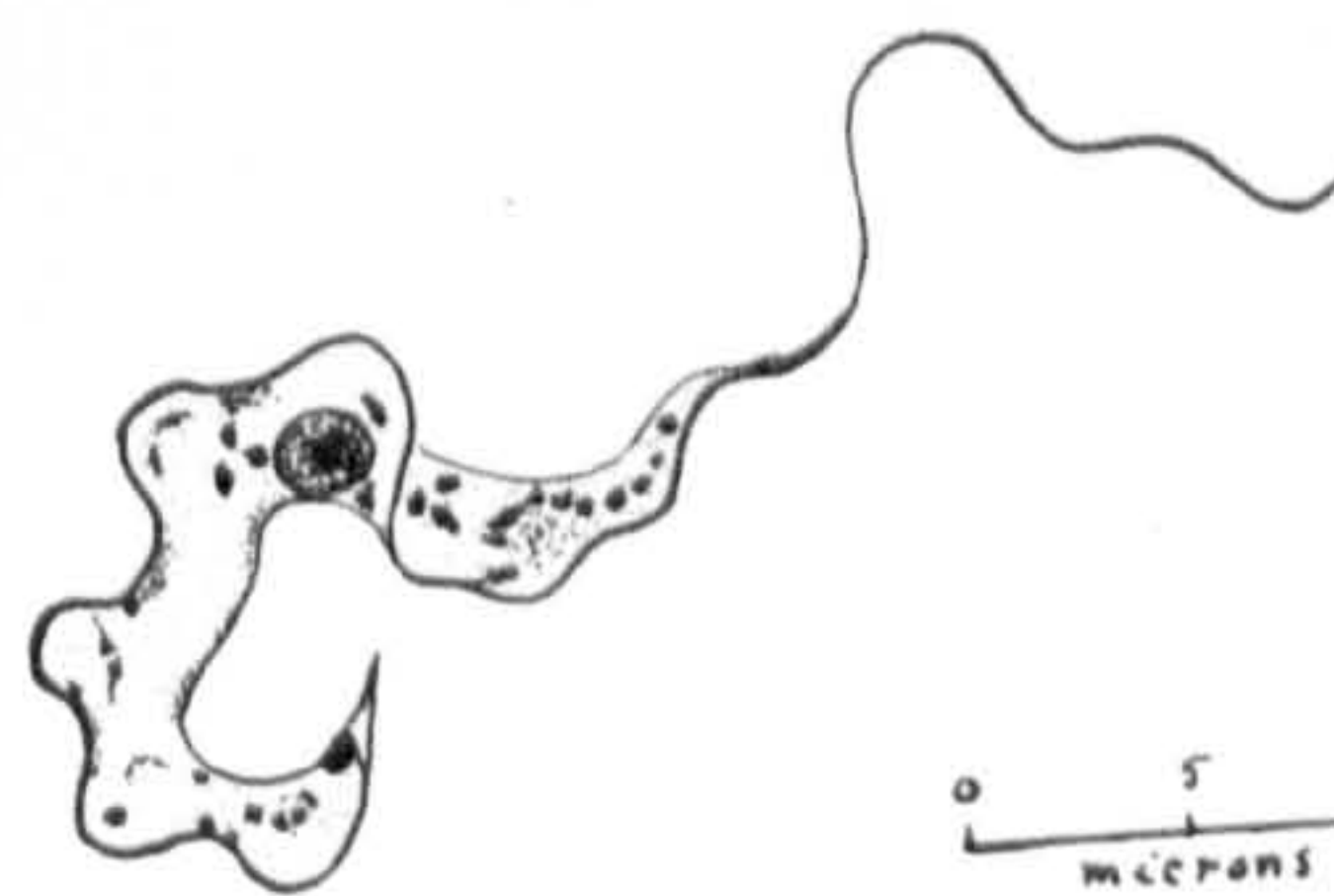
112



113



114



115

0 5 10  
microns



Plate No. VII.

Cultural forms of T. winchii.

- Figs. 116 to 118. Crithidial tadpole-like forms with different shapes of nuclei.
- Fig. 119. The same with small body and flagellum.
- Fig. 120. Sub-globular form which appears leishmania like.
- Figs. 121 to 125. The same.
- Fig. 126. Stumpy form with rod-shaped parabasal.
- Fig. 127. Crithidial form; the parabasal and nucleus at the posterior end of the body with well developed undulating membrane.
- Fig. 128. The same with karyosome and vacuolated ring.
- Figs. 129 & 130. The same; large vacuoles at the posterior end of the body.
- Fig. 131. The same; parabasal and nucleus connected with a cord.
- Fig. 132. Stumpy form.
- Fig. 133. Crithidial form.
- Fig. 134. Stumpy form with rhizoplast.
- Fig. 135. The same.
- Fig. 136. Small mature trypanosome form.
- Fig. 137. Dividing form; two newly formed karyosomes without connecting line.
- Fig. 138. Globular form. The same condition as in Fig. 137.
- Fig. 139. Dividing forms. Haematoxylin stain has been extracted much to see if there are chromosomes to be seen but no chromosome were found.

Plate VII



116



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118



119



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124



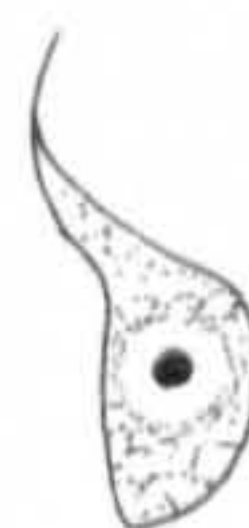
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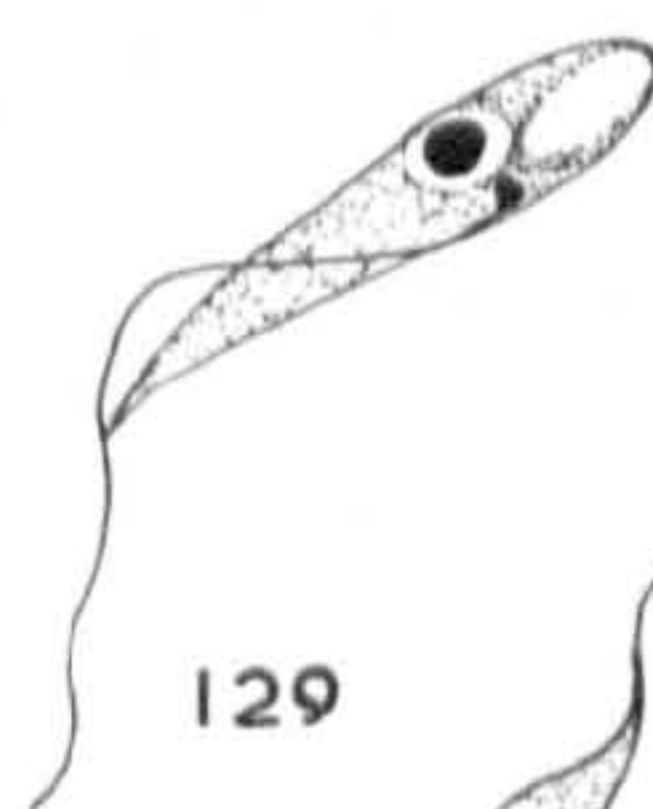
126



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129



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131



132



133



134



135



136



137



138



139





Plate No. VIII.

Cultural forms of T. winchii

- Fig. 140. Dividing form. The two daughter parabasals with two new nuclei.
- Fig. 141. Crithidial form; nucleus divided before the division of parabasal body.
- Fig. 142. The same; parabasal small, rod-shaped.
- Fig. 143. The same; division of parabasal.
- Fig. 144. Dividing form: the band shaped nucleus after the division of parabasal.
- Fig. 145. The same; spindle shaped nucleus.
- Fig. 146. The same; nucleus connected with a long cord, simulating a drum stick.
- Fig. 147. The same; nucleus long and band shaped.
- Fig. 148. The same; two unequal nuclei.
- Fig. 149. The same; nuclei connected with a long cord.
- Figs. 150,  
151 & 152. The same.
- Fig. 153. The same; two daughter parabasals with two new nuclei.
- Fig. 154. The same; constriction of the cytoplasm.
- Figs. 155  
to 157. Small mature trypanosome forms with short flagellum.
- Fig. 158. Attenuated crithidial form with long flagellum.
- Fig. 159. Stumpy multiple type with four nuclei.

Plate VIII



140



141



142



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147



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149



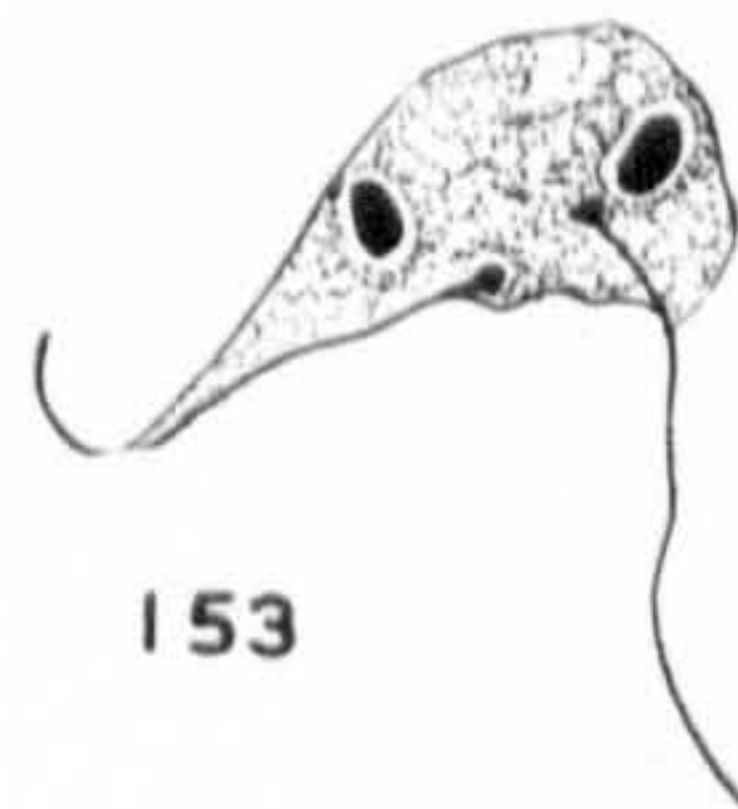
150



151



152



153



154



155



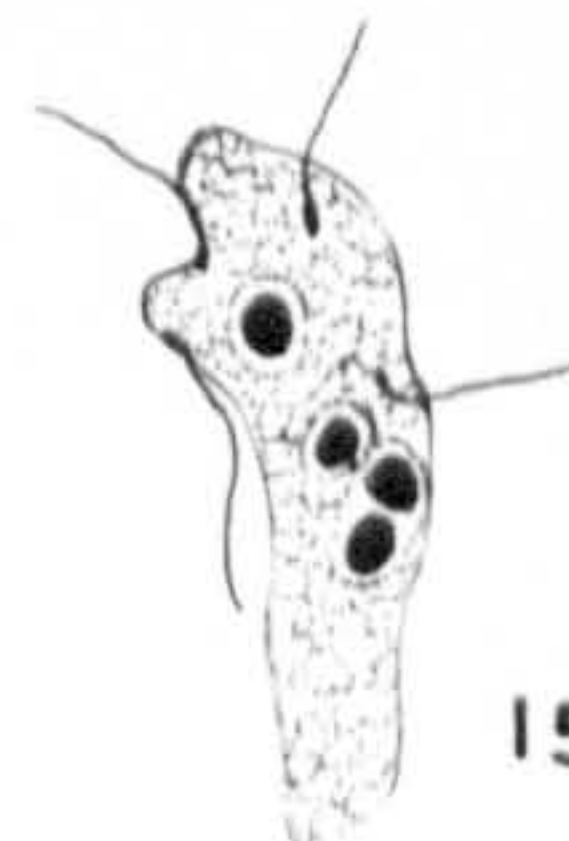
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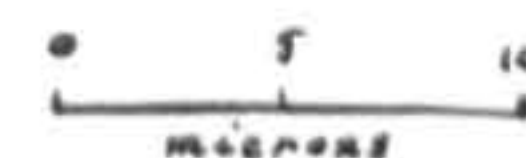
157



158



159





T. danilewskyi in the blood of carp and their  
transmission in the leech Hemiclepsis marginata.

- Figs. 160 to 167. Blood trypanosome forms.
- Figs. 168 to 169. T. danilewskyi in the crop of leech.  
Smears made three hours after feeding.
- Fig. 170. The same; five hours after feeding.
- Figs. 171 to 175. The same; one day after feeding.
- Figs. 171 & 172. The same; crithidial forms.
- Figs. 173 to 175. The same; dividing forms.
- Fig. 175. The same; crithidial form in section  
of crop of leech.
- Figs. 176 & 177. The same; crithidial form two days  
after feeding.
- Fig. 178. The same; dividing form.
- Figs. 179 to 186. The same; crithidial and dividing  
forms three days after feeding.

Plate ix

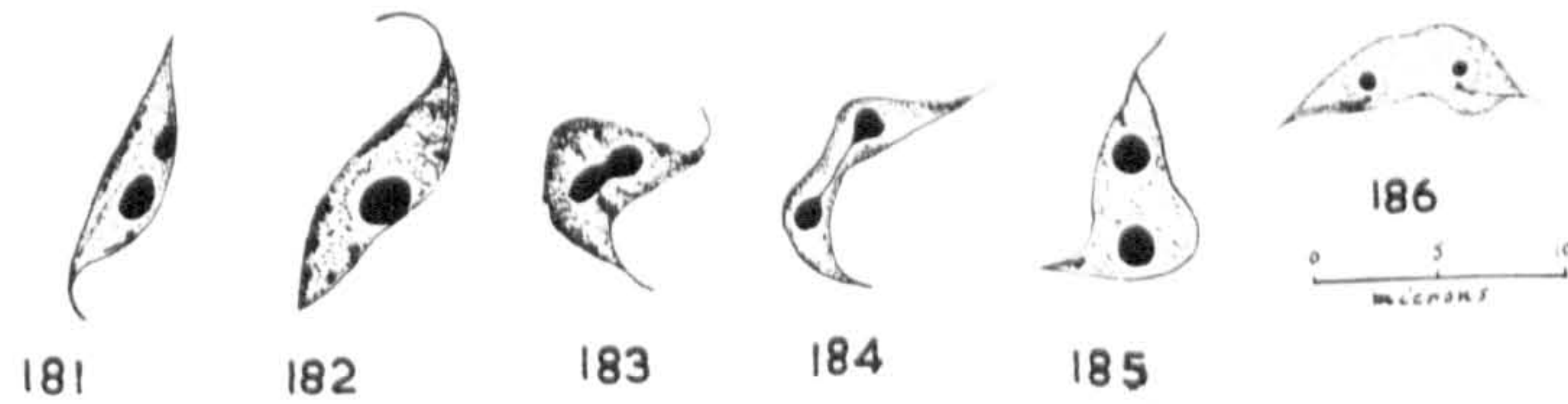
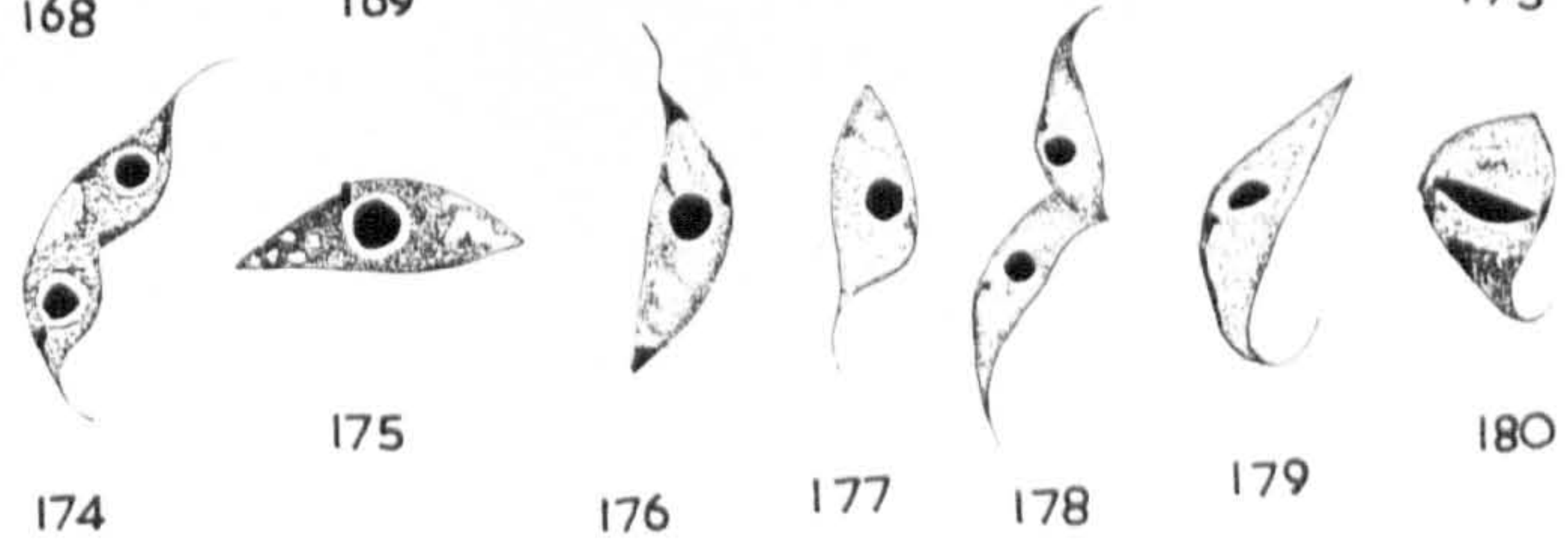
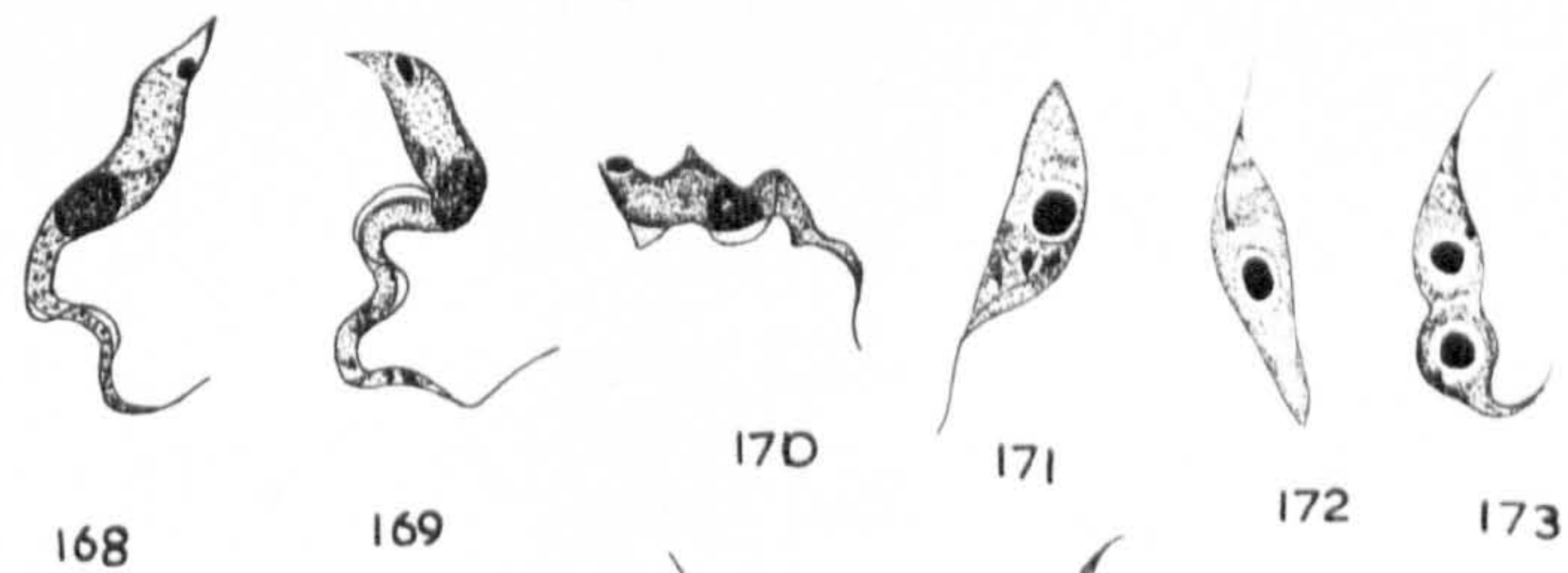
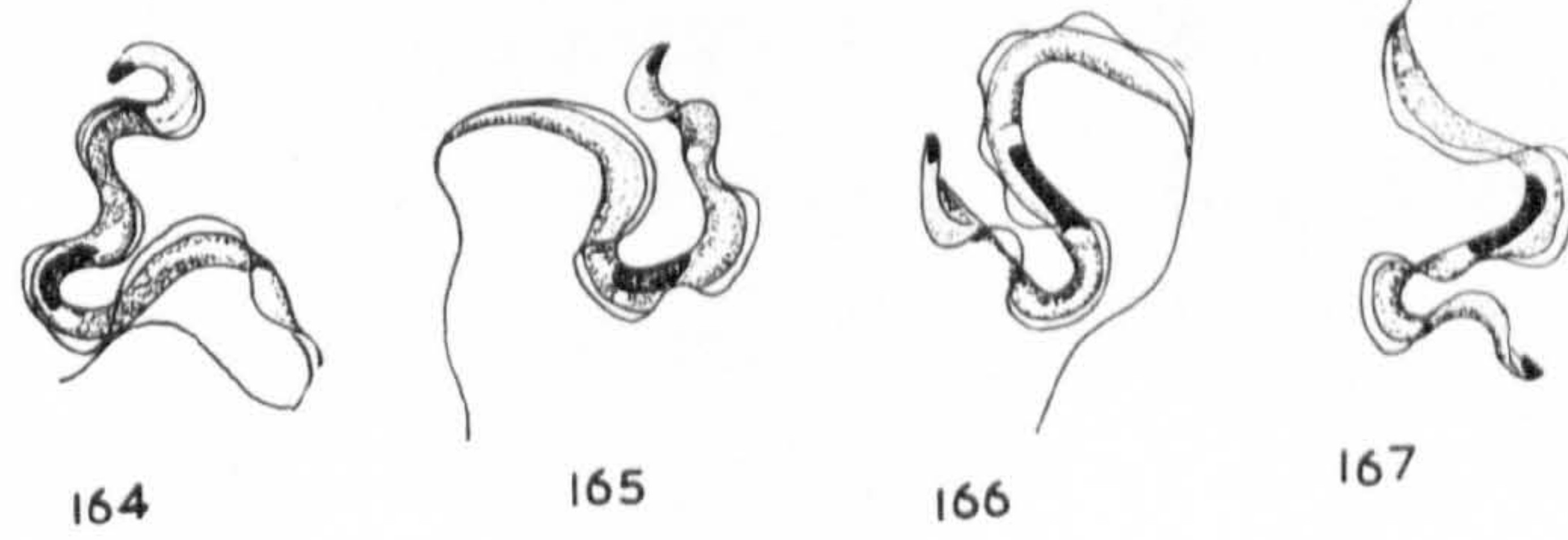
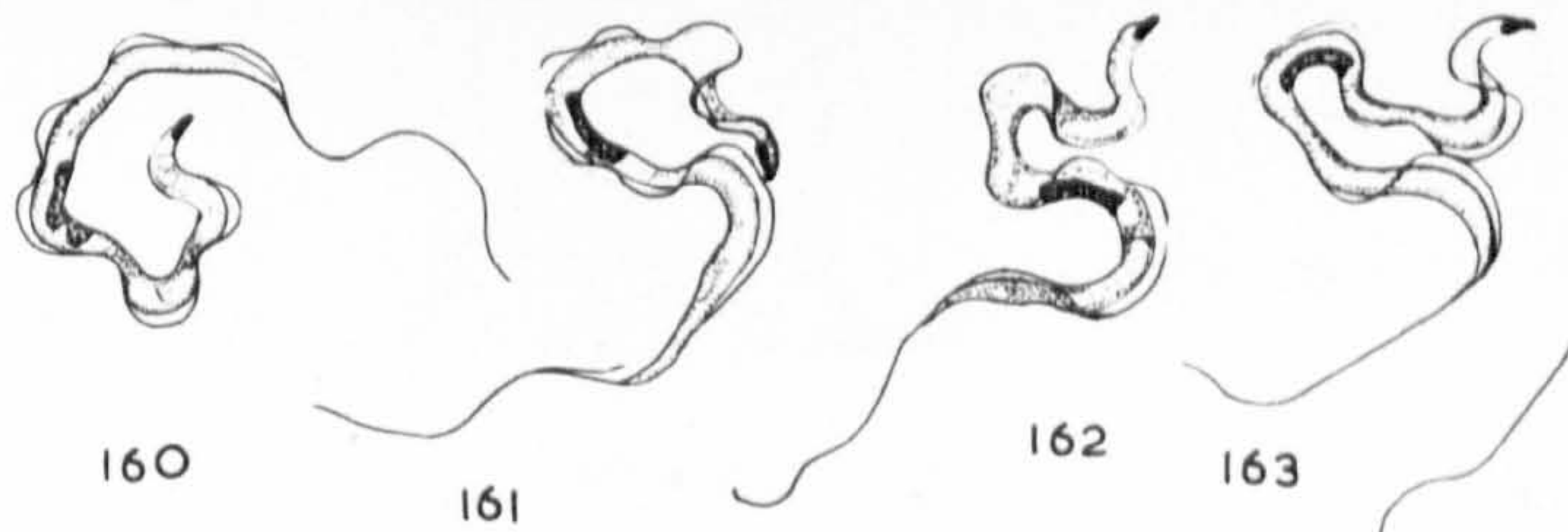




Plate No. X.

Transmission of T. danilewskyi of carp  
in the leech, Hemiclepsia marginata.

- Figs. 187 to 195. Stages of trypanosome in the crop of leech four days after feeding.
- Figs. 187, 190, 191. Leptomonas forms.
- Figs. 188, 189, 192, 194, 195. Crithidial forms.
- Fig. 193. Dividing form.
- Figs. 196 to 204. Slender forms of trypanosome in the leech five days after feeding.
- Figs. 196, 197, 202 & 203. Crithidial slender forms.
- Fig. 198. Dividing form.
- Figs. 199, 201, & 204. Crithidial tadpole like forms.
- Figs. 205 to 212. Slender and dividing trypanosome forms in the crop of leech six days after original feed.
- Fig. 205. Crithidial tadpole like form.
- Fig. 210, 211. Dividing form.
- Figs. 213 to 219. Small and large attenuated forms of trypanosome seven days after feeding.
- Fig. 216. Small crithidial form.

Plate x

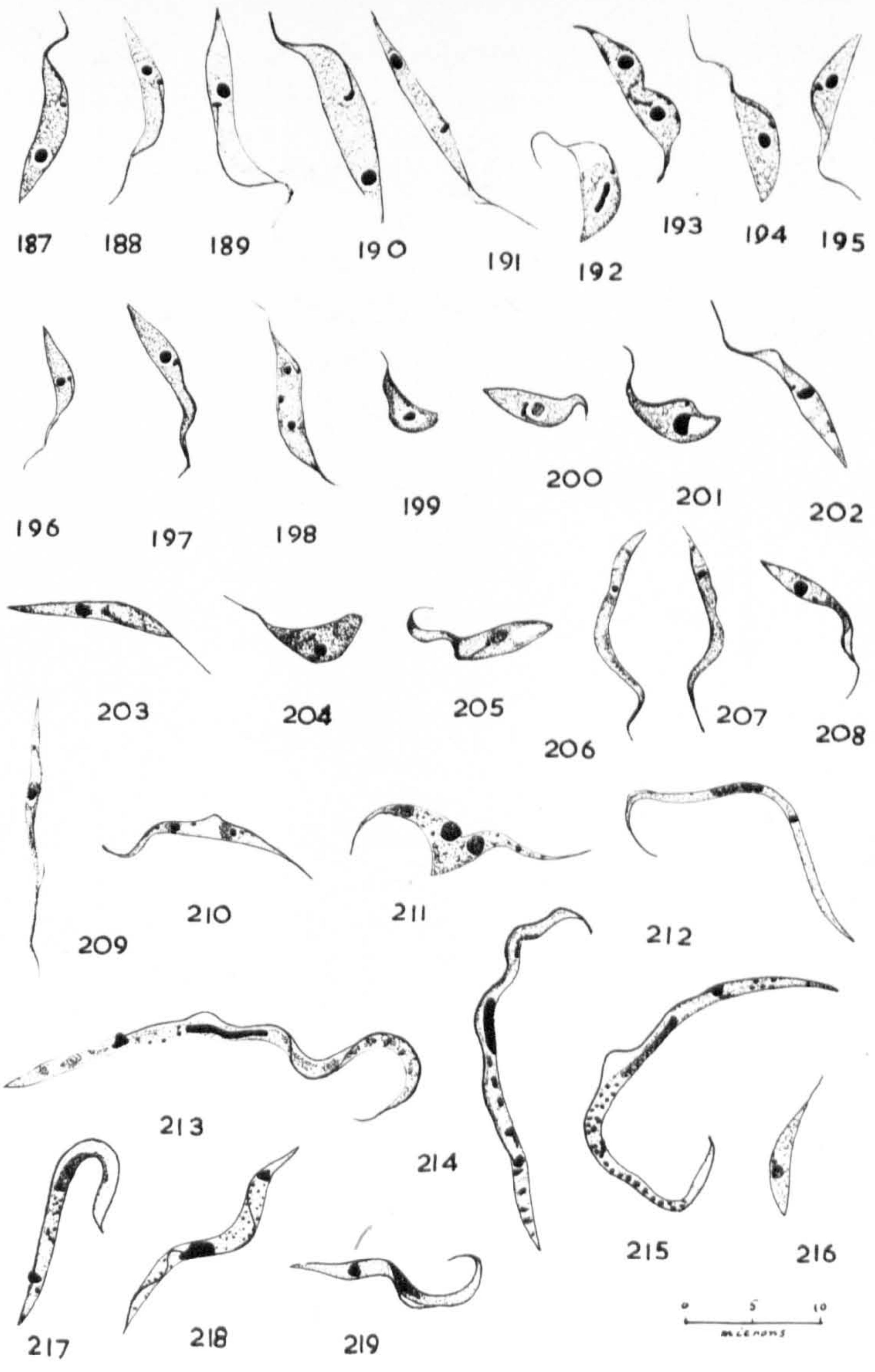




Plate No. XI.

Transmission of T. danilewskyi of carp  
in the leech Hemiclepsis marginata.  
Films stained with Giemsa stain.

Figs. 220, 221. Small and intermediate slender forms  
of trypanosome, seven days after feeding.

Figs. 222 to  
226. Slender trypanosomes from the proboscis  
sheath of leech, on 8th day after  
feeding.

Figs. 227 to  
230. Small and large attenuated trypanosomes  
from the proboscis sheath on 9th day  
after feeding.

Figs. 231 to  
236. Trypanosoma percae from the blood of  
perch, Perca fluviatilis.

Plate xi

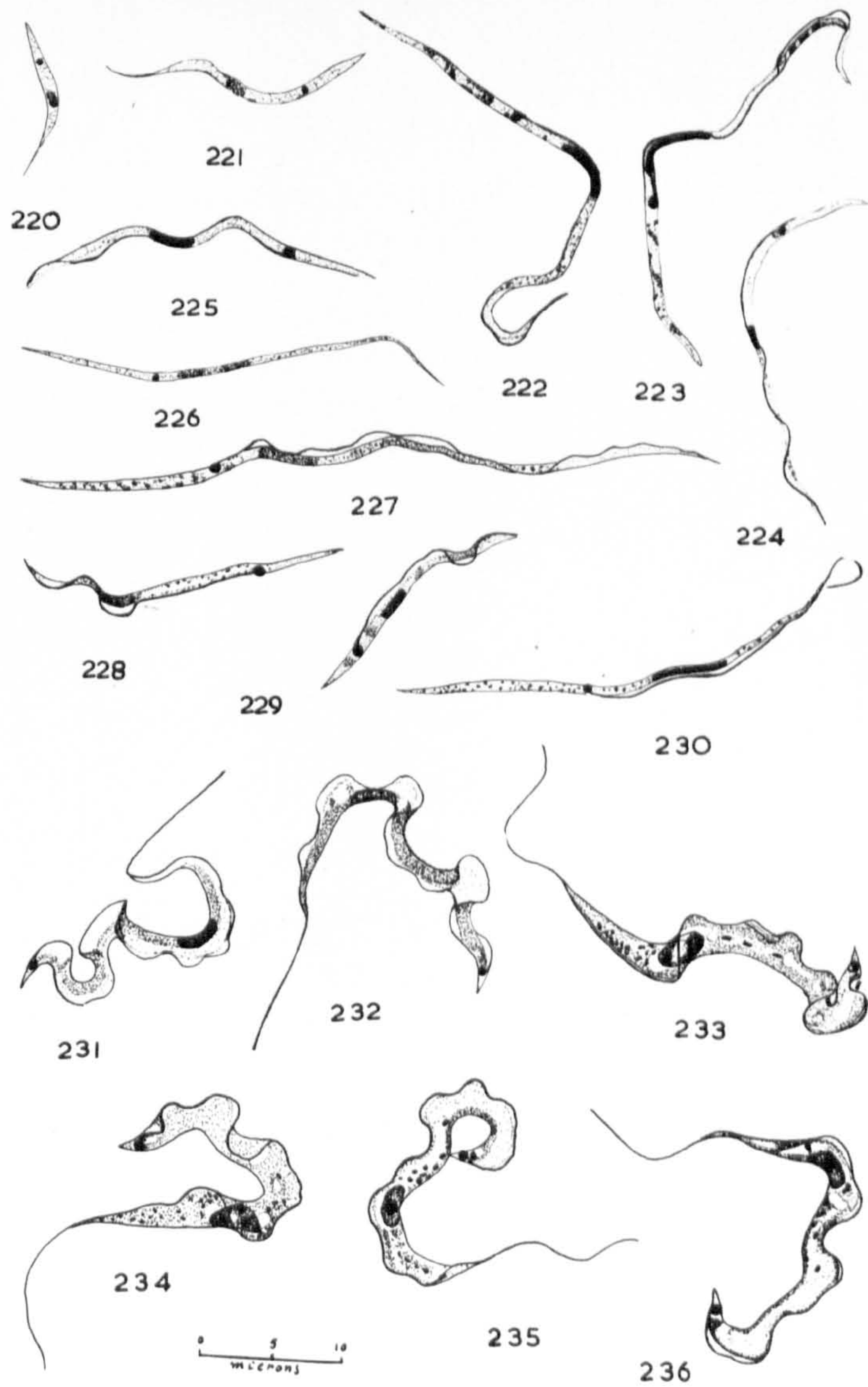




Plate No. (B)

Life cycle of Trypanosoma danilewskyi in  
the blood of fish and in the leech.

- Figs. 1 & 2. Trypanosomes in the blood of Carp.
- Figs. 3 to 18. Developmental stages of trypanosomes  
in the crop of leech.
- Fig. 3. Trypanosomes in blood of fish; form  
ingested by leech. (3 hours after  
feeding).
- Fig. 4. Leptomonas form.
- Figs. 5 to 7. Crithidial forms, in the crop.
- Figs. 8 to 12. Dividing forms.
- Figs. 13 to 14. Young crithidial forms.
- Figs. 15 to 18. Long attenuated forms in the proboscis  
sheath of the leech.

Plate ( B )

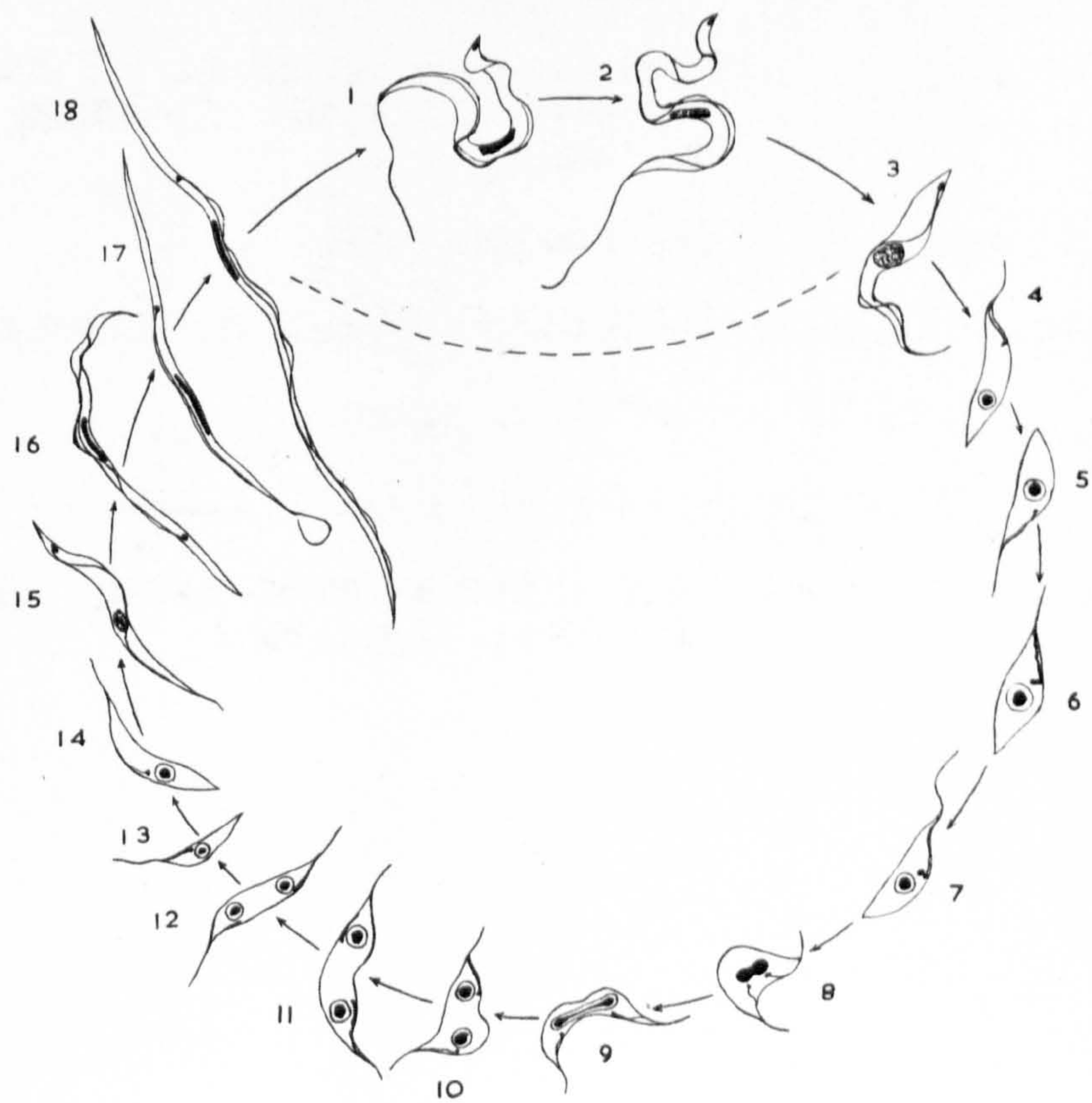




Plate No. XII.

These figures are all stained with Giemsa stain  
and are drawn with the aid of camera lucida.

Figs. 237 to 245. Trypanoplasma willoughbii in the blood  
of char.

Figs. 237, 240  
to 242 & 245. Large forms.

Figs. 238, 239  
& 244. Intermediate forms.

Figs. 243. Small form.

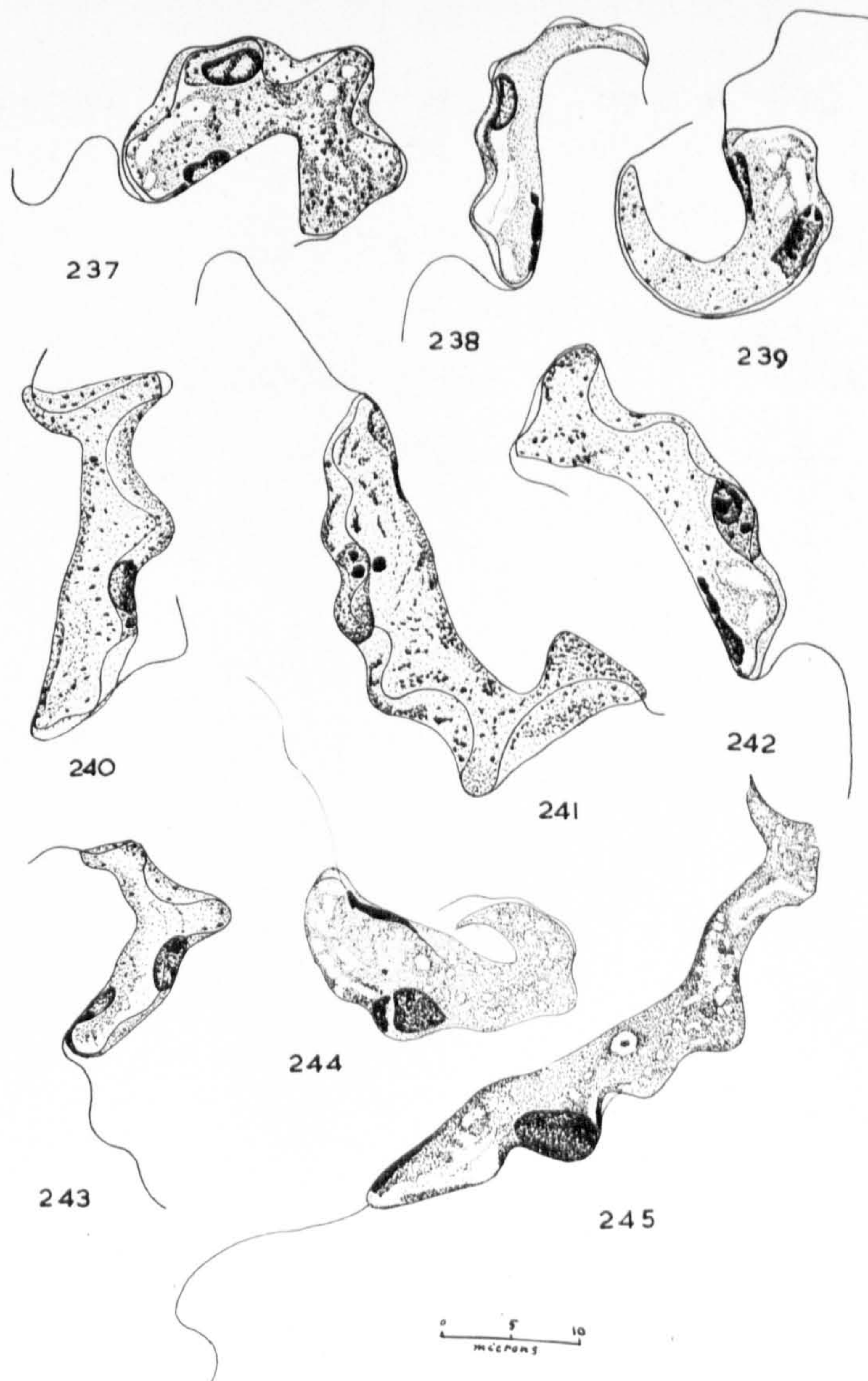




Plate No. XIII.

Cultural forms of Tp. willoughbi.

- Figs. 246 & 247. Fresh specimens drawn after using osmic vapour and methylene blue. Many volutin granules are present and the nuclear membrane is distinct.
- Figs. 248 to 254. Slender trypanoplasm forms.
- Fig. 255. Squat form.
- Fig. 256. Small irregular form.
- Figs. 257, 259. Small oval shaped forms.
- Fig. 260. Slender form with posterior end swollen.
- Fig. 261. Small "Bodo" like form.
- Figs. 262, 264. Small forms with broad undulating membrane.
- Fig. 263. Stumpy form with many flagella arising from one parabasal body.
- Figs. 265 to 269. Small and large forms.
- Fig. 268. Stumpy form with nucleus division.

Plate XIII

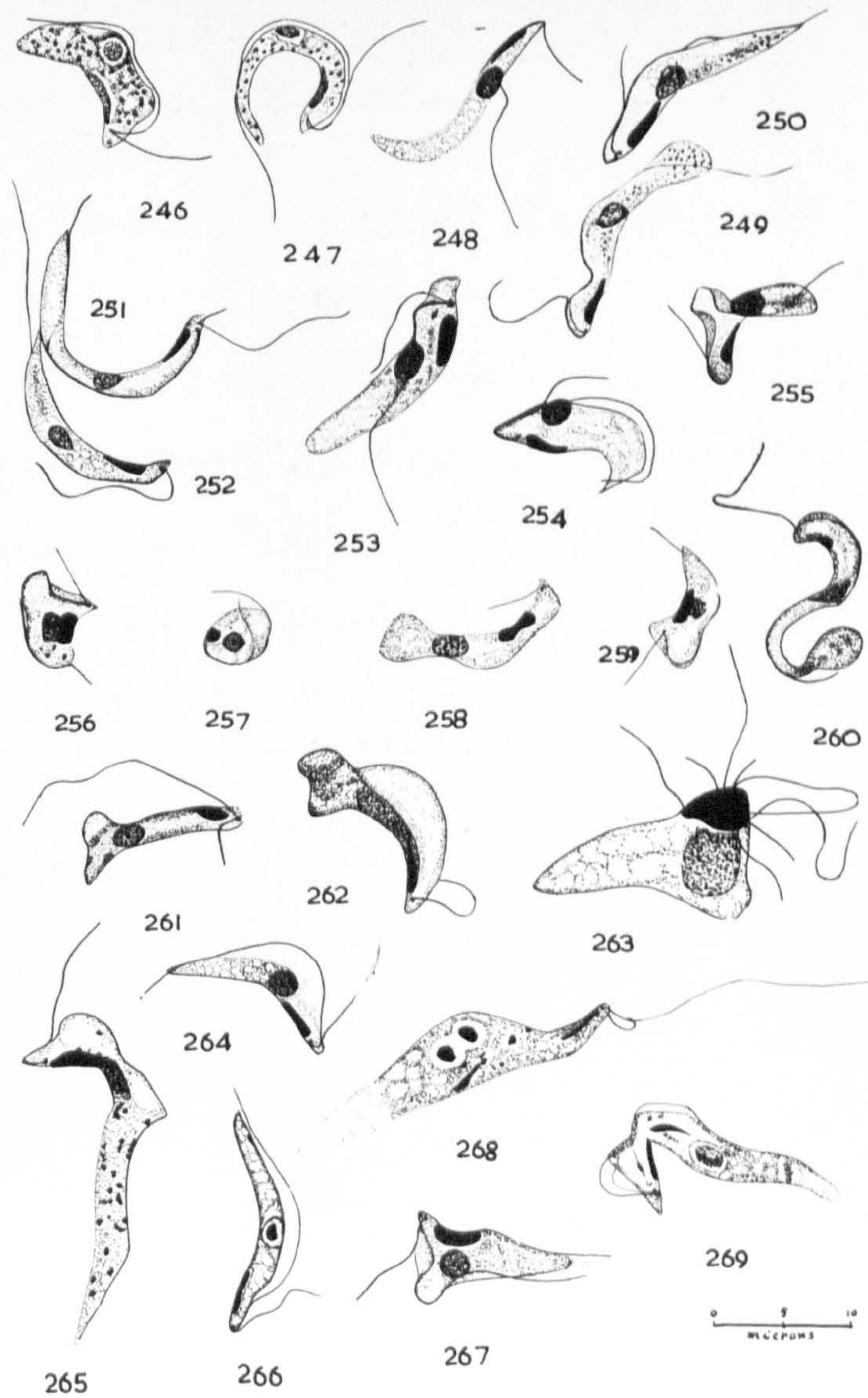




Plate No. XIV.

Cultural forms of Tp. willoughbii in culture.  
Stained with Giemsa stain.

- Figs. 270, 280. Dividing forms with two parabasal bodies and two nuclei.
- Fig. 279. Dividing form with two newly formed parabasal bodies at one end of the body and two nuclei at the other.
- Fig. 281. Small form.
- Fig. 282. Multiple fission with many small forms.
- Fig. 283. The same with four small forms.

Plate xiv

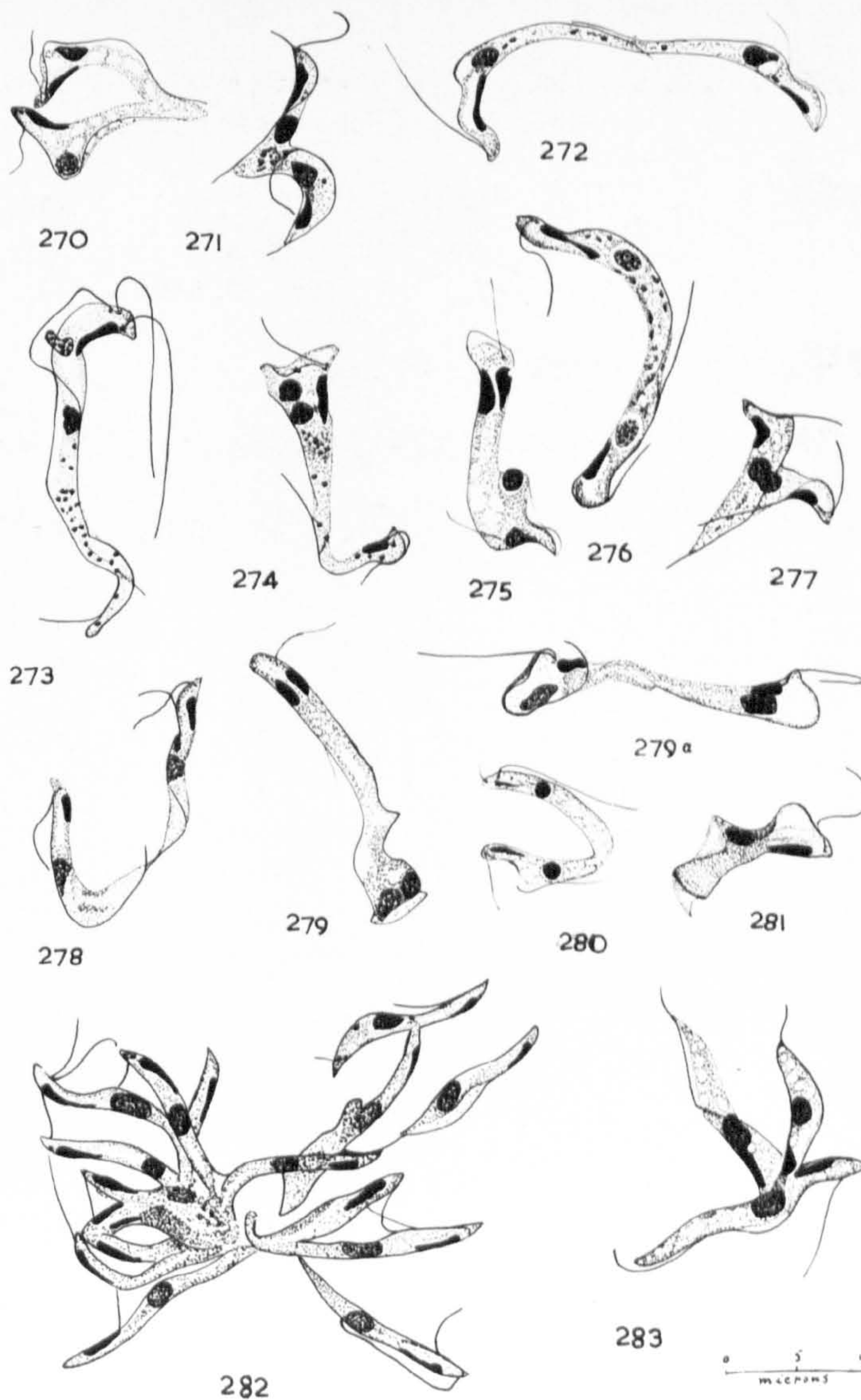




Plate No. XV.

Trypanoplasma guernei in the blood of  
bullhead. Stained with Giemsa.

Fig. 284. Small form.

Figs. 285, 288. Intermediate forms.

Fig. 287. Small slender form.

Figs. 286, 289  
to 294. Large forms with volutin granules.

plate xv

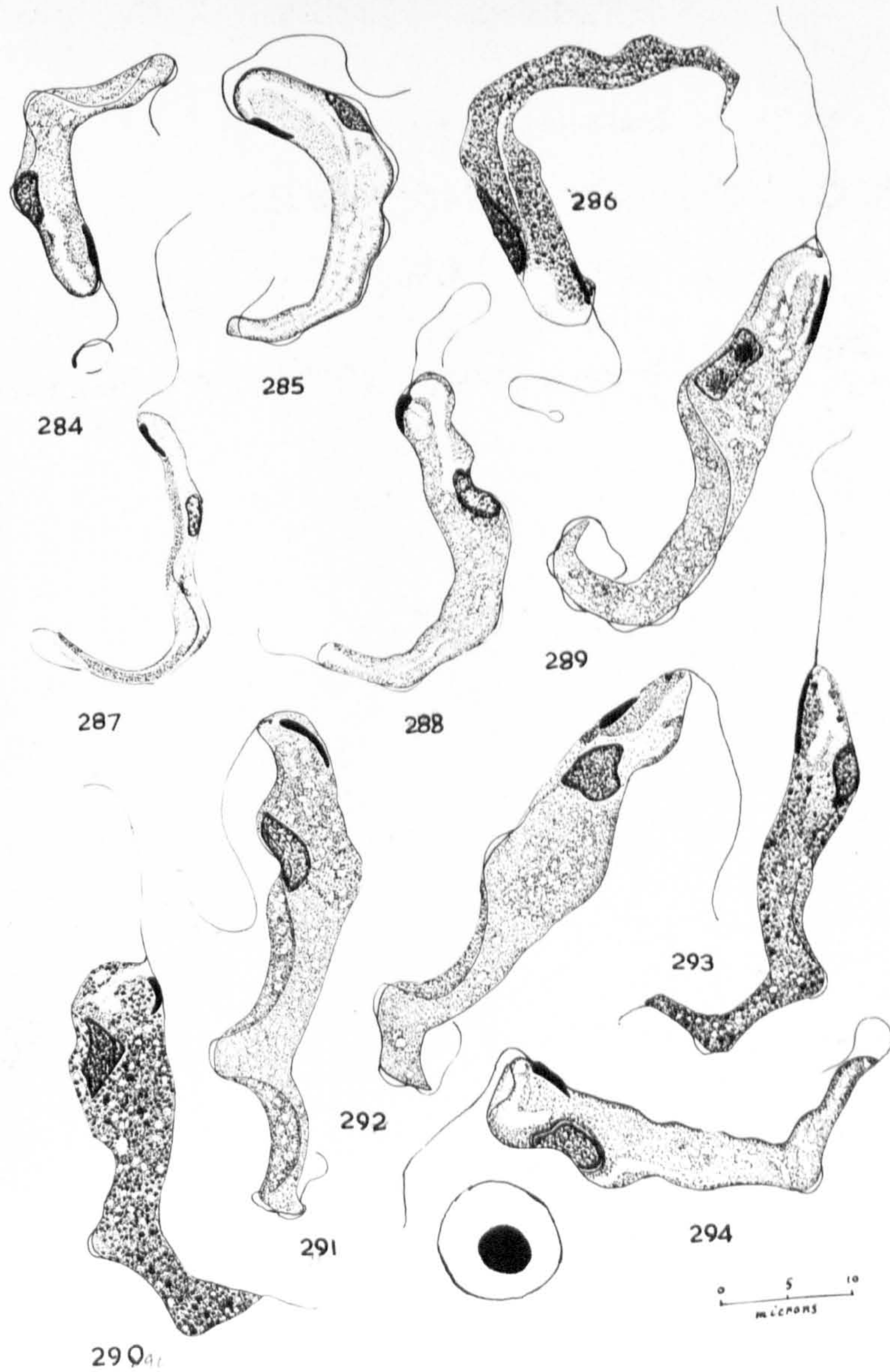




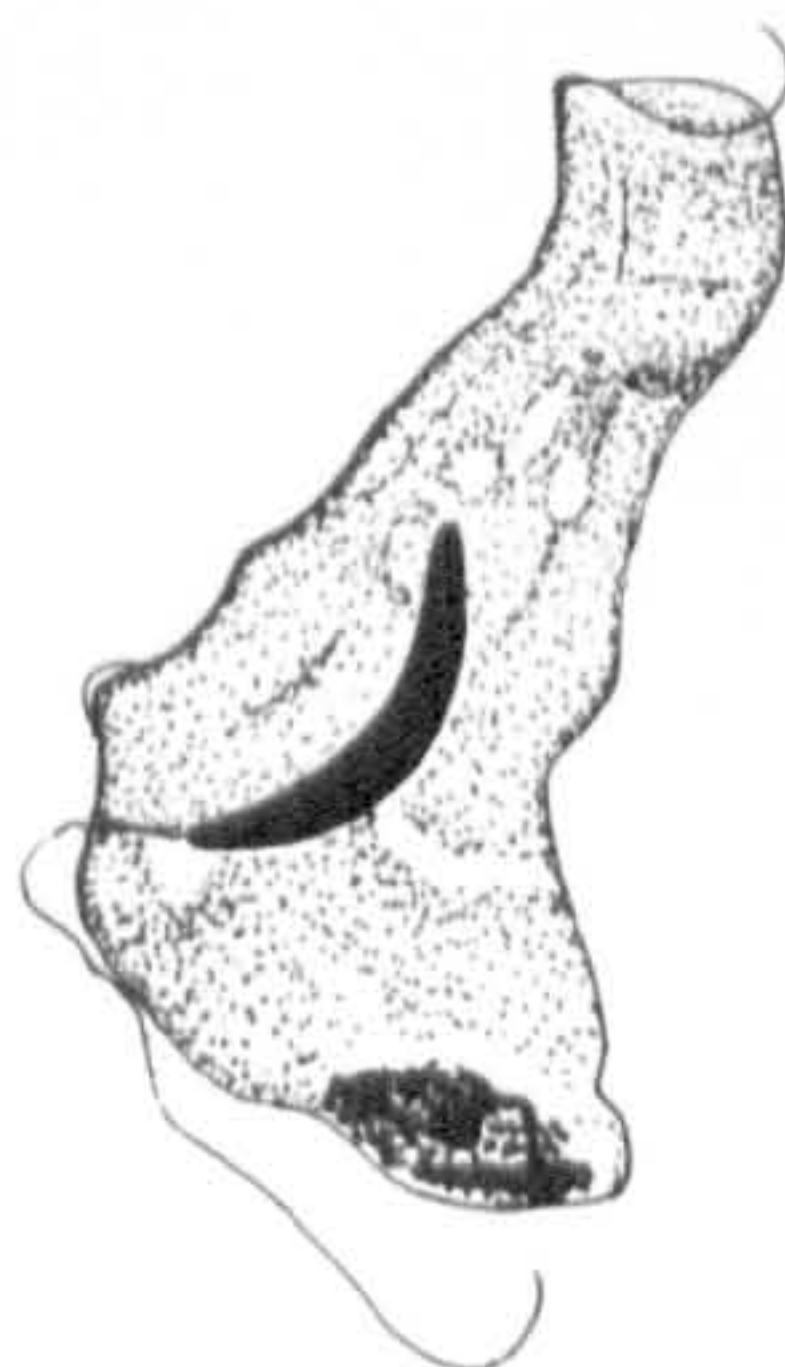
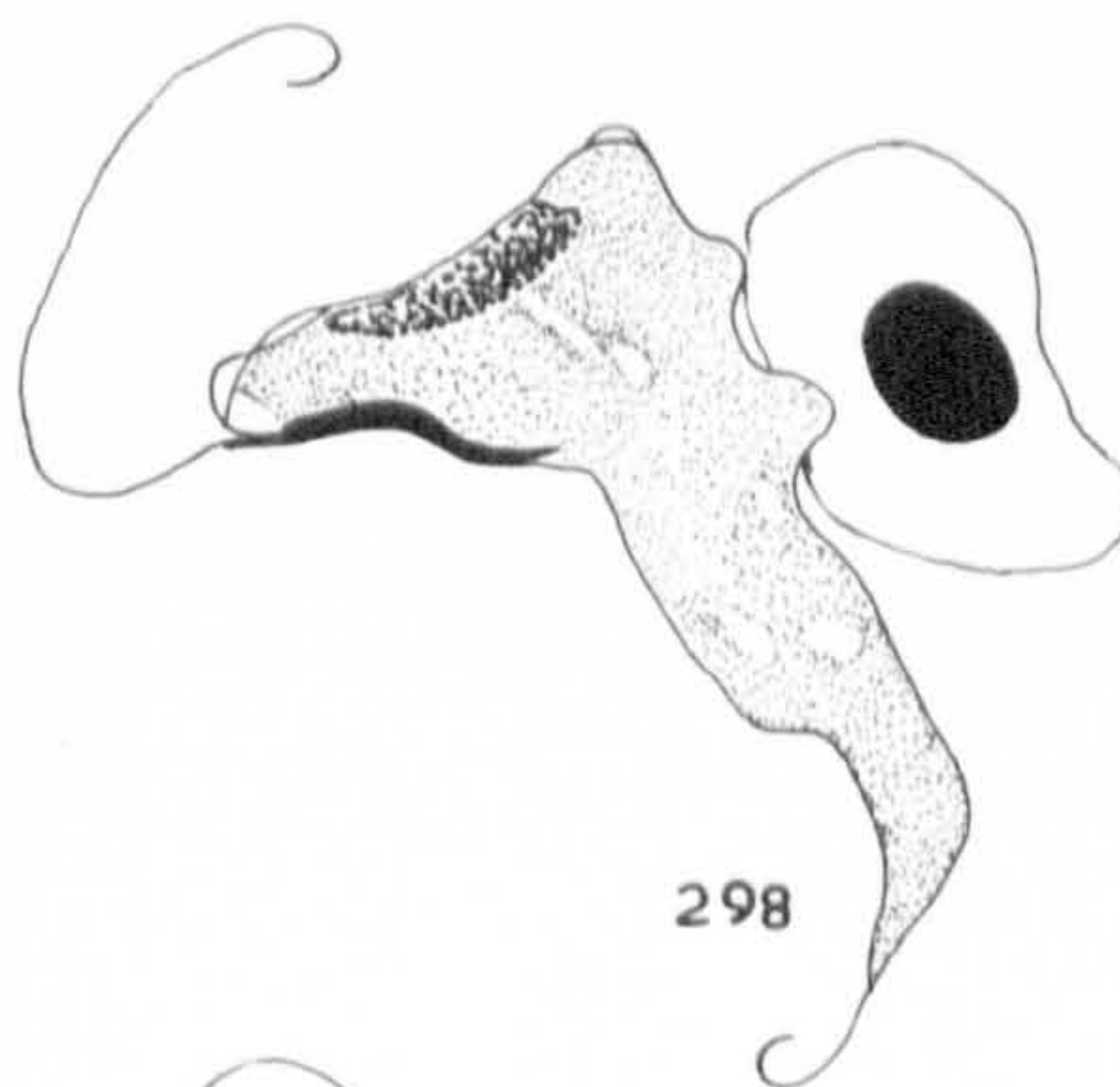
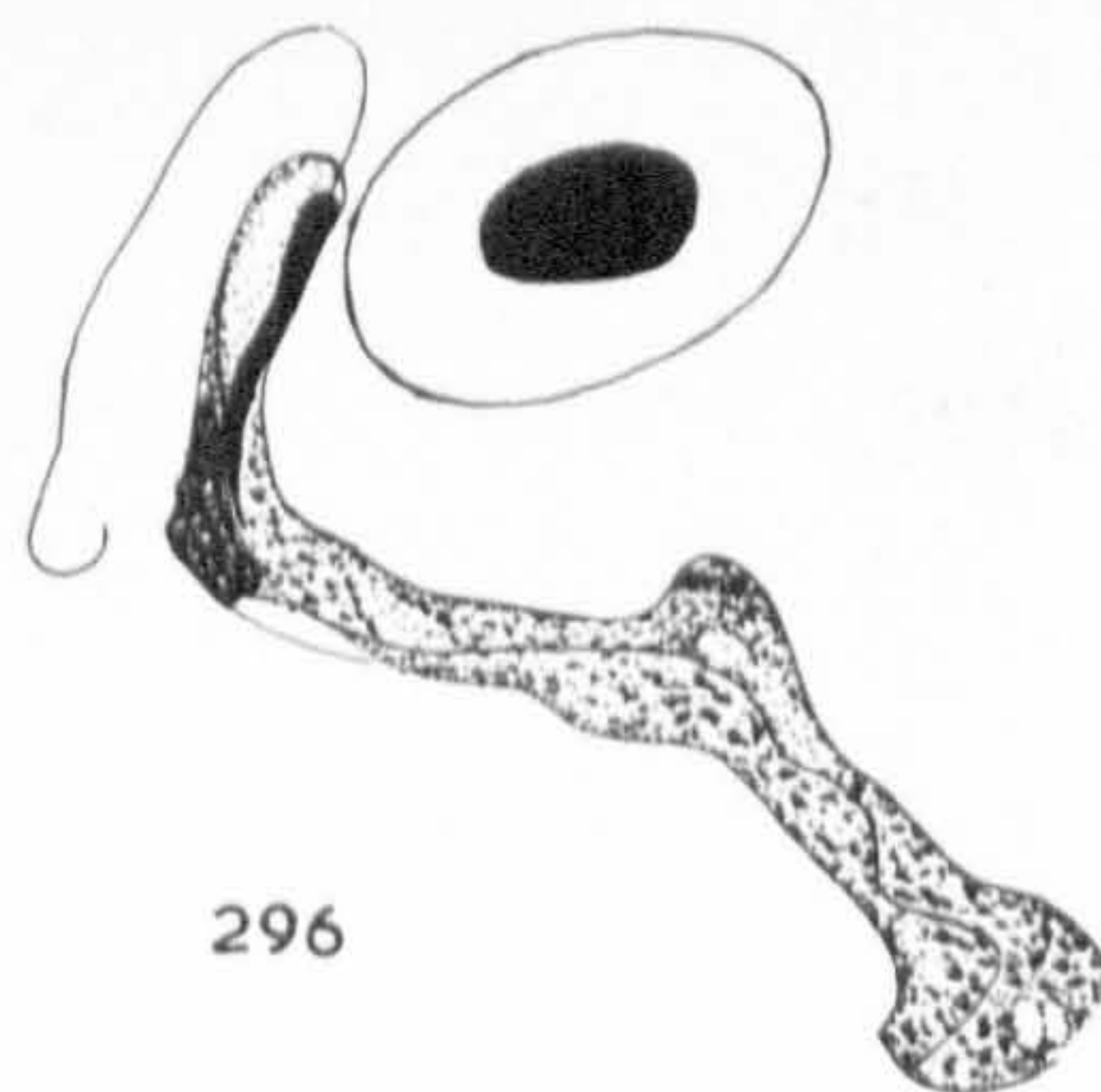
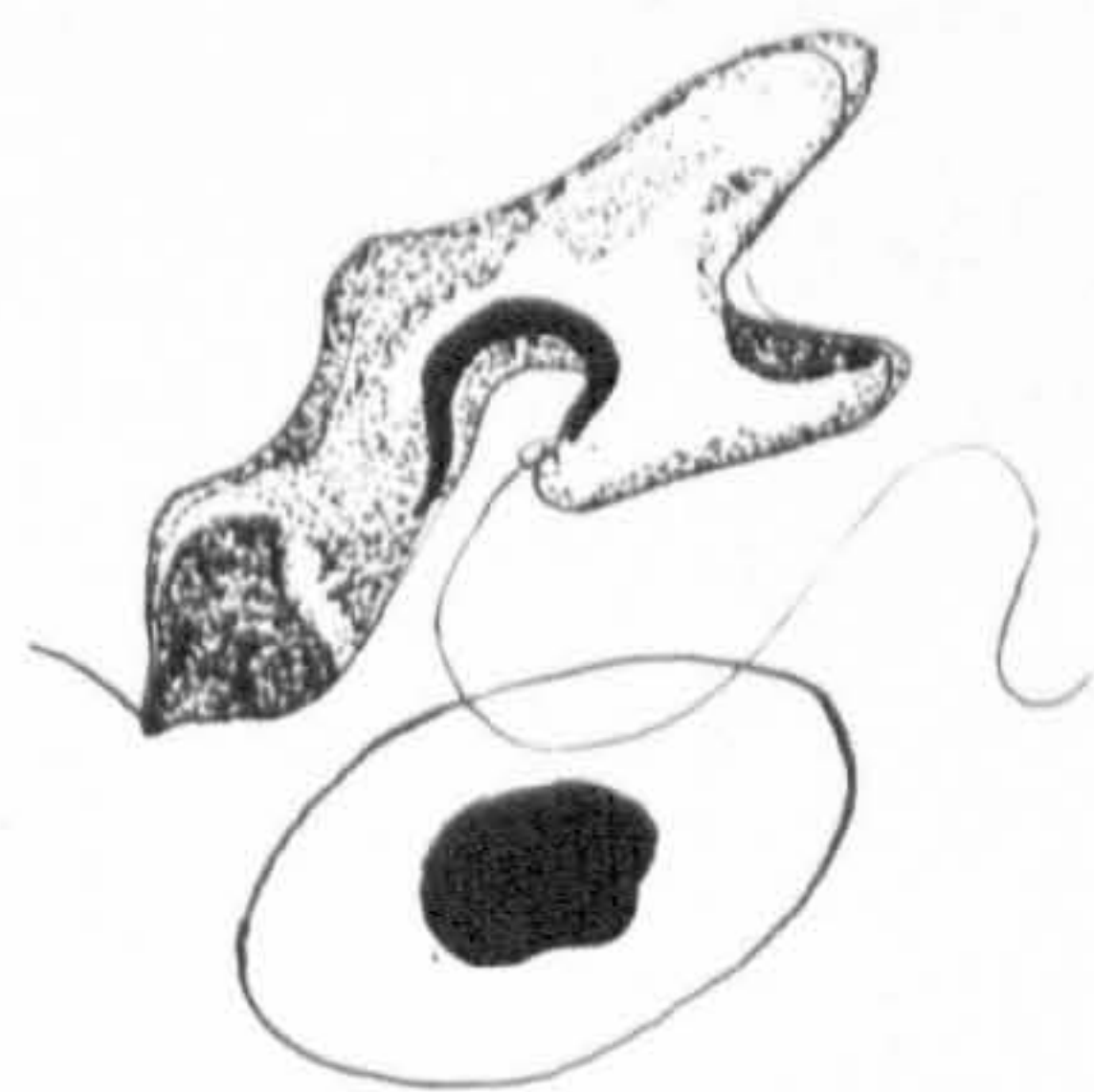
Plate No. XVI.

Trypanoplasma cyprini from the blood  
of carp.

Figs. 295 to 300. Large forms.

Fig. 296. Large slender form with volutin  
granules.

Plate xvi



0 5 10  
microns



Plate No. LVII

Trypanosoma danilewskyi var clarii and  
Trypanosoma danilewskyi var saccobranchi  
in the blood of Clarias batrachus and  
Saccobranchus fossilis, Indian fish.

Figs. 301 to 309. T. danilewskyi var clarii.

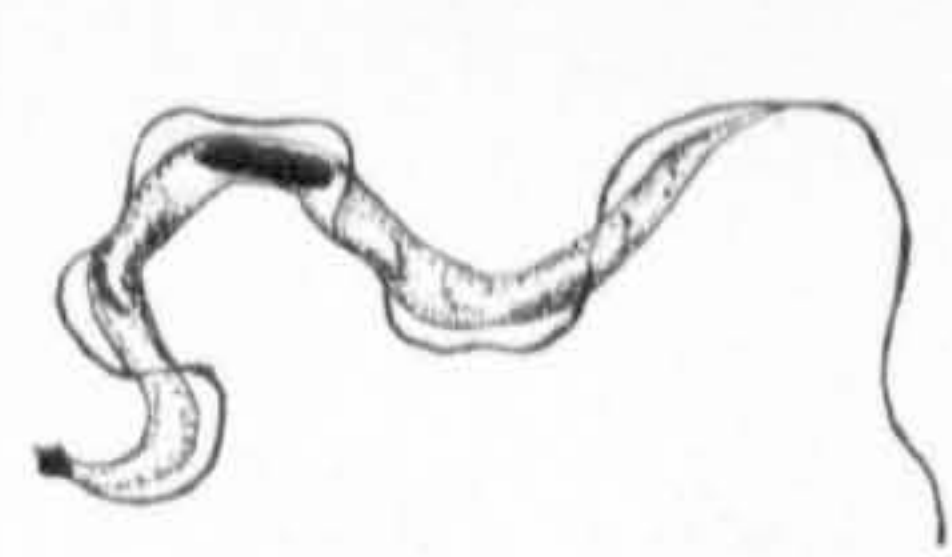
Figs. 301 to 304  
& 309. Large forms. (Giemsa stain .

Fig. 305. Large form with nuclear division.

Figs. 307 & 308. Stumpy forms.

Figs. 310 to 317. T. danilewskyi var saccobranchi.  
Large forms.

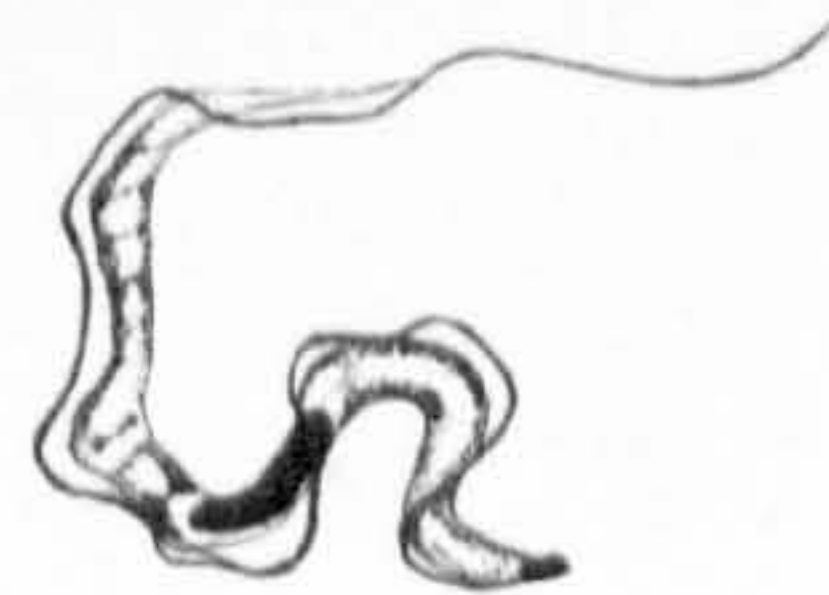
Plate xvii



301



302



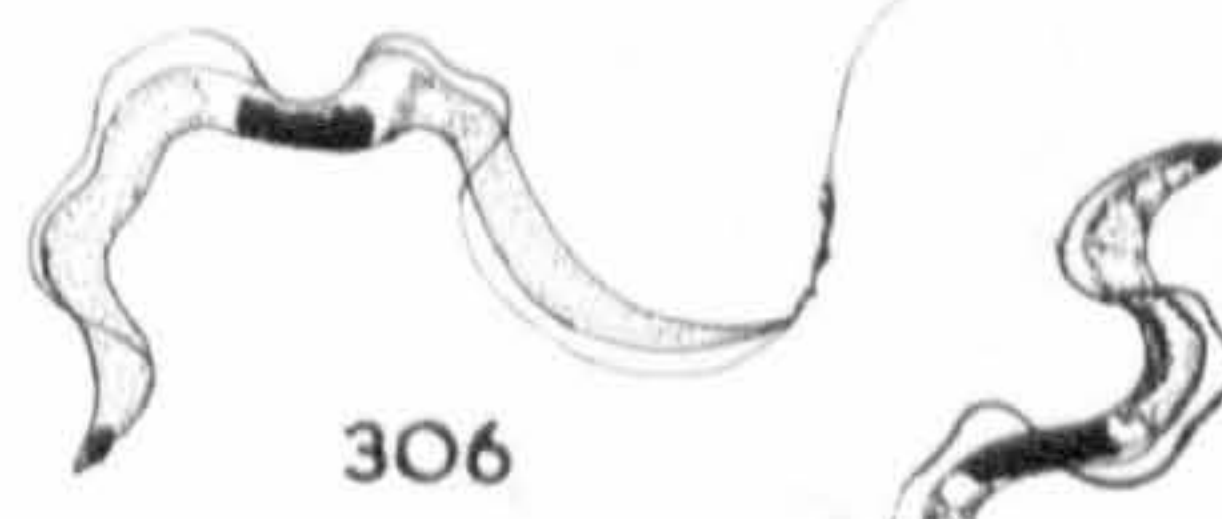
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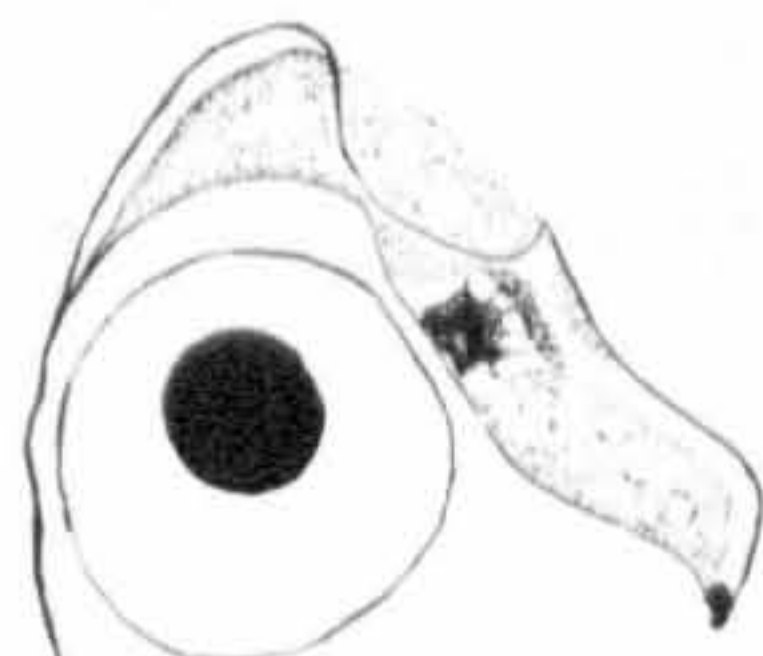
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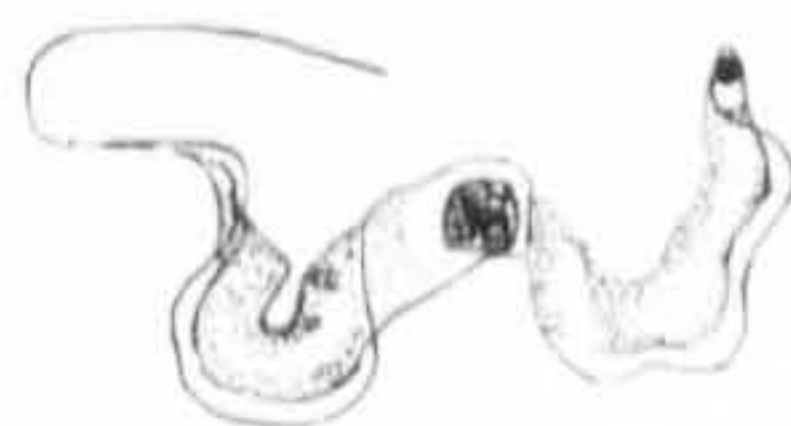
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306



307



308



309



310



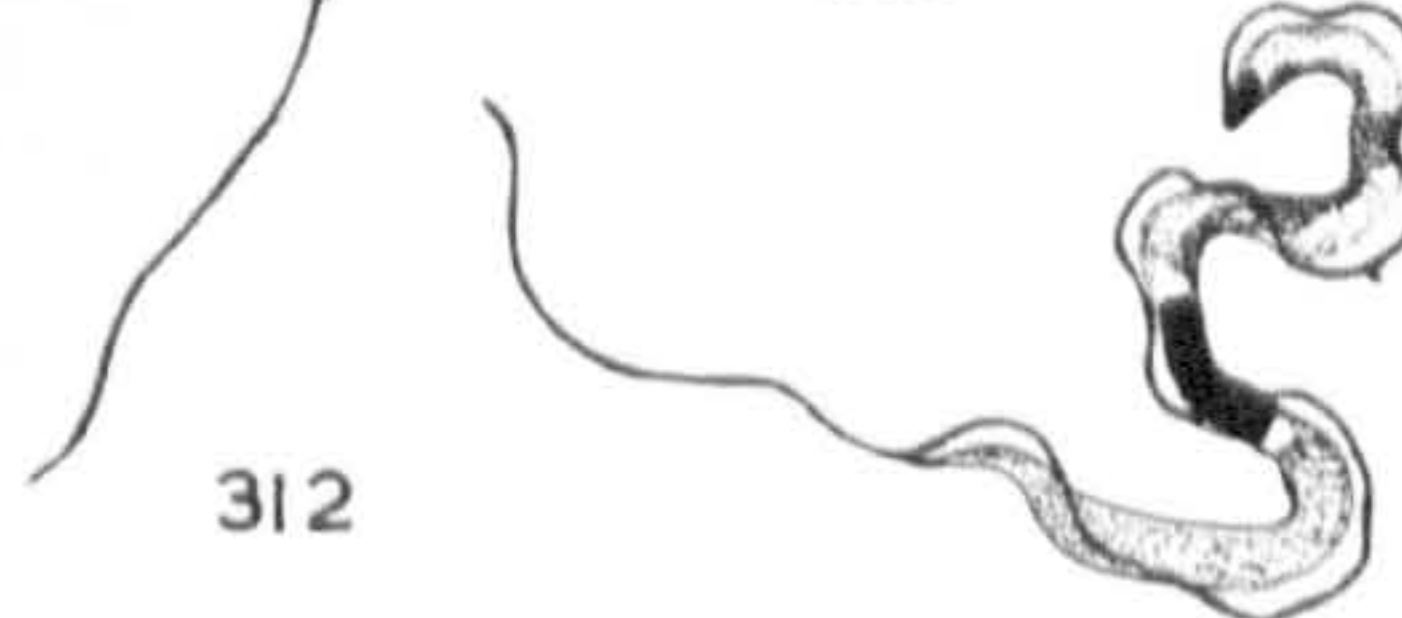
311



312



313



314



315



316



317



0 5 10  
microns



Plate No. XVIII.

Trypanosoma abramidis from the blood of  
breast. They are all stained with Giemsa  
stain.

Figs. 318 to 326  
& 228, 229.

Small and large forms.

Fig. 327.

Trypanosomic form in culture made by  
placing a drop of blood on a sterile  
slide, covering with cover glass and  
examining three days after.

Figs. 330 to 333.

Cultural forms obtained from the  
same slide mentioned above.

Figs. 330 & 331.

Crithidial tadpole-like forms.

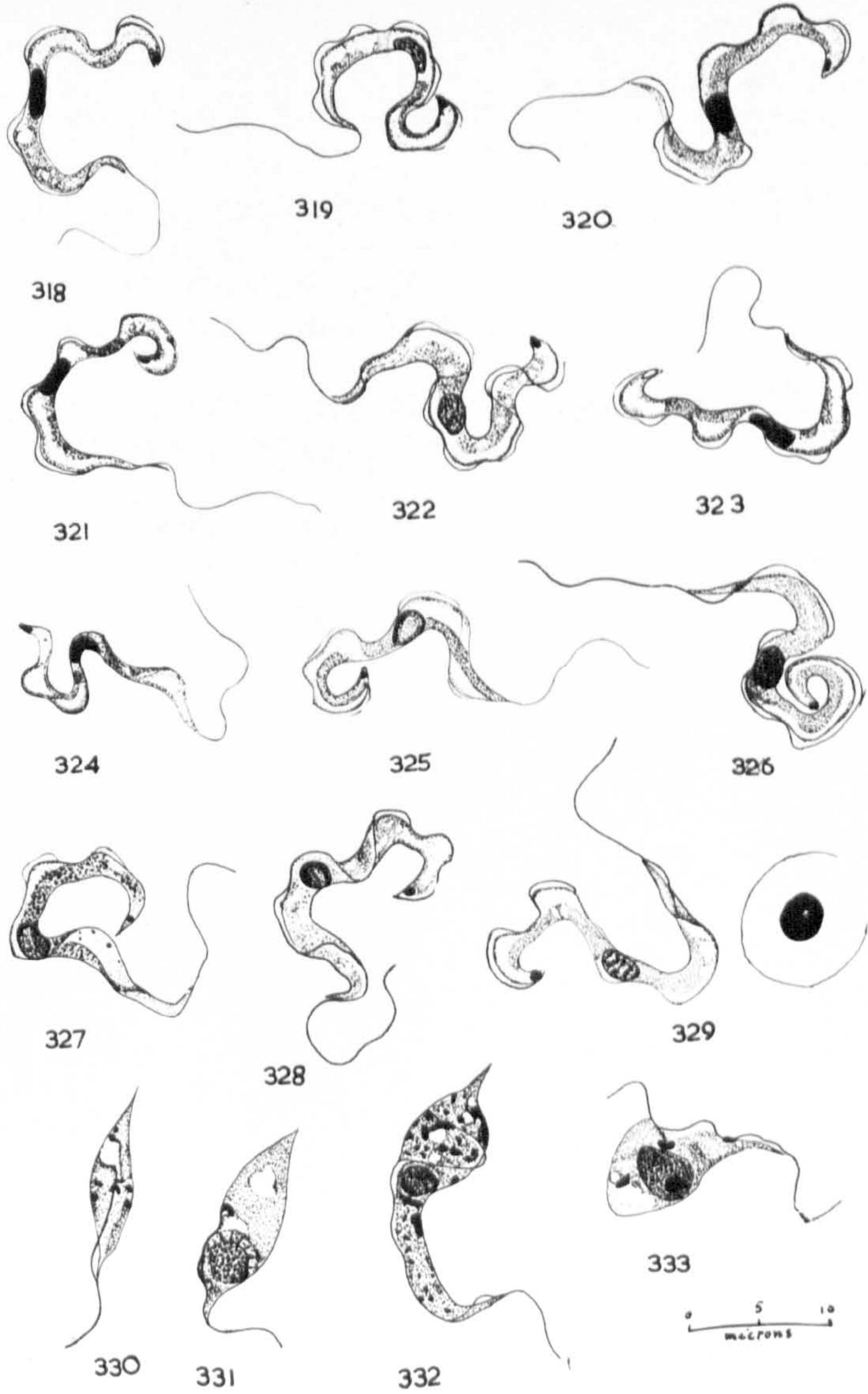
Fig. 332.

The same with the posterior body  
in swollen condition.

Fig. 333.

Dividing form.

Plate XVIII





Myxobolus clarii from Clarias batrachus,  
Indian fish. Giemsa stain.

- Figs. 334 & 335. Uninucleate pansporoblasts.
- Figs. 336 to 340. Binucleate pansporoblasts.
- Figs. 341 to 343. Tetranucleate pansporoblasts.
- Figs. 344 to 347.  
249 & 255. Multinucleate pansporoblasts.
- Figs. 350 to 373. Spores of Myxobolus clarii.
- Figs. 350 to 352. Spores with sutural ridges.
- Fig. 354. Spore showing two polar capsules  
one at each pole.
- Figs. 357 to 364  
& 366 to 370. Spores with developed capsules, parietal  
nuclei, capsulogenous nuclei,  
sporoplasmic nuclei and iodophilous  
vacuoles, and two polar capsules.
- Fig. 362. Spore with three polar capsules.
- Fig. 356. Portion of intestine of fish with  
nodules of . clarii.
- Fig. 365. Section of nodules in liver. Earlier  
stages are near the periphery and the  
developed spores in the middle of the  
nodule.
- Figs. 371 & 372. Large spores with long pyriform  
polar capsules.
- Fig. 373. Spore with two small polar capsules.

Plate xix

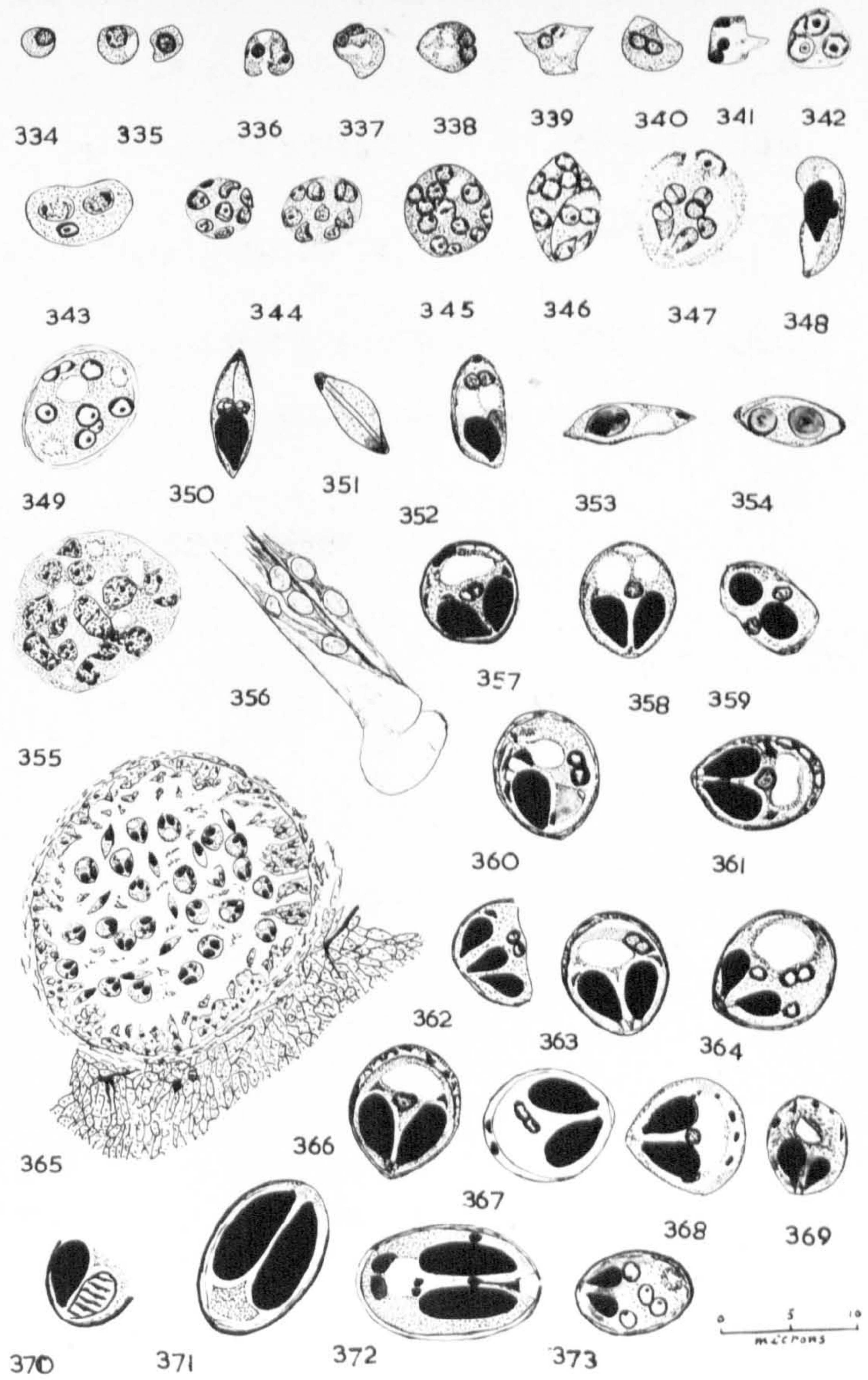




Plate No. XX.

- Figs. 374 to 380. Myxobolus clarii from Clarias batrachus, an Indian fish.
- Figs. 374 to 376. Spores with three polar capsules.
- Fig. 377. Spores with four polar capsules.
- Figs. 378 to 380. Spores with extruded polar filaments.
- Figs. 381 to 401. Myxidium scardini n.sp. from Scardinius erythrophthalmus (Rudd). Stained with Giemsa stain.
- Figs. 381 & 382. Uninucleate pansporoblasts.
- Fig. 383. Binucleate pansporoblast.
- Figs. 384 & 385. Tetranucleate pansporoblasts.
- Fig. 388. Multinucleate pansporoblast.
- Figs. 386 & 390. Front view of the spores.
- Figs. 387, 391 to 399. Developed spores with sutural line, capsulogenous nuclei, sporoplasmic nuclei and capsules, one at each pole.
- Figs. 400 to 401. Spores with extruded polar filaments.

Plate xx

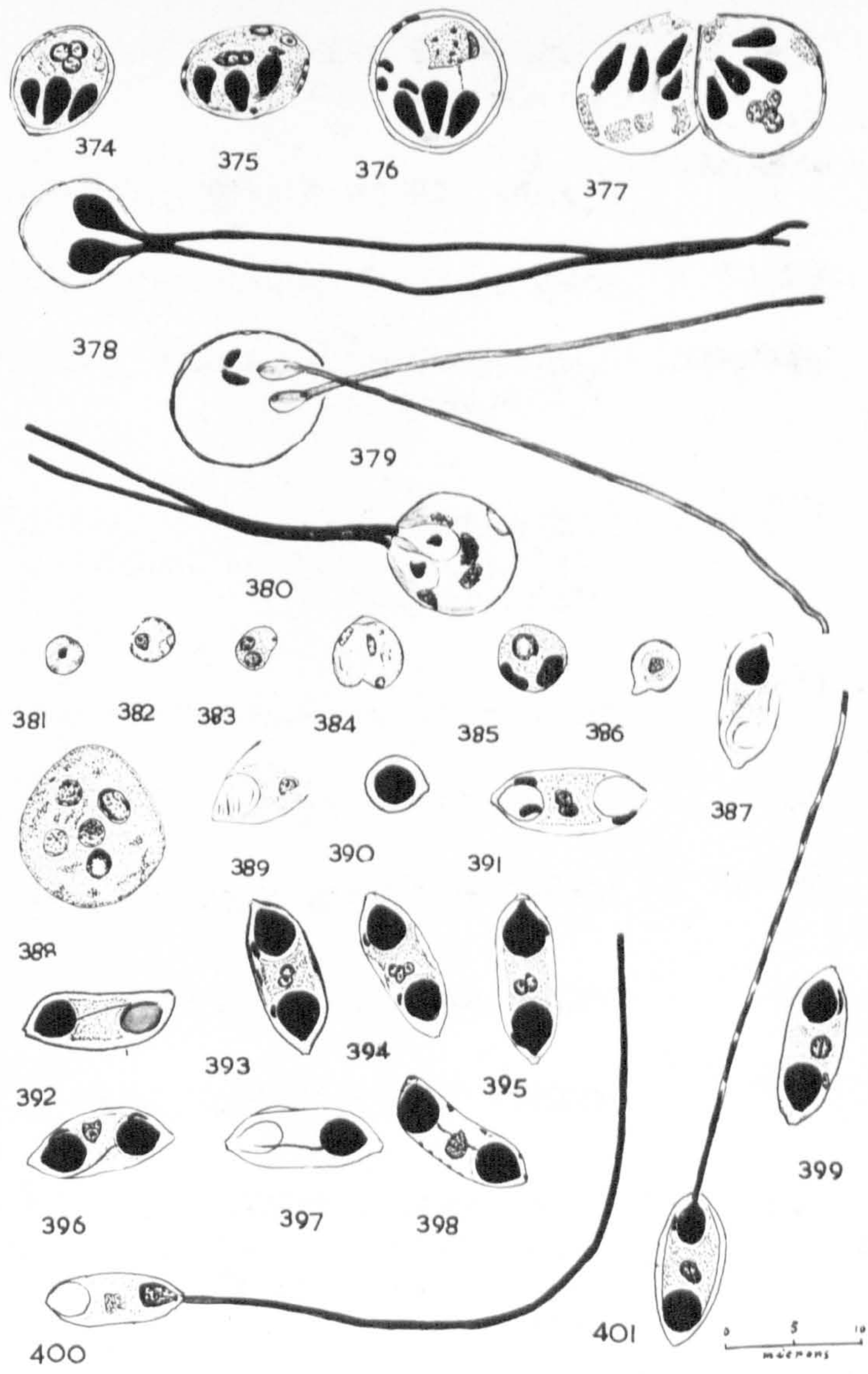




Plate No. XXI.

Zschokkella cyprini n.sp from Cyprinus carpio. Giemsa stain. All figures drawn from the section of the bile duct in liver.

Figs. 402 to 403. Uninucleate pansporoblasts.

Figs. 404 to 405. Binucleate pansporoblasts.

Figs. 406 to 409. Tetranucleate pansporoblasts.

Fig. 410. Multinucleate pansporoblast.

Figs. 411 to 417. Development of two sporoblasts within the sporont.

Fig. 418. Sporont with two developed spores.

Figs. 419 to 422. Fully developed with capsulogenous and sporoplasmic nuclei and polar capsules one at each pole.

Fig. 423. Fully extruded polar filament.

Figs. 424 to 430. Henneguya psorospermica from Perca fluviatilis (perch). Giemsa stain.

Fig. 424. Immature spore.

425 to 430. Spores fully formed, with extrusion of polar filaments.

Plate XXI

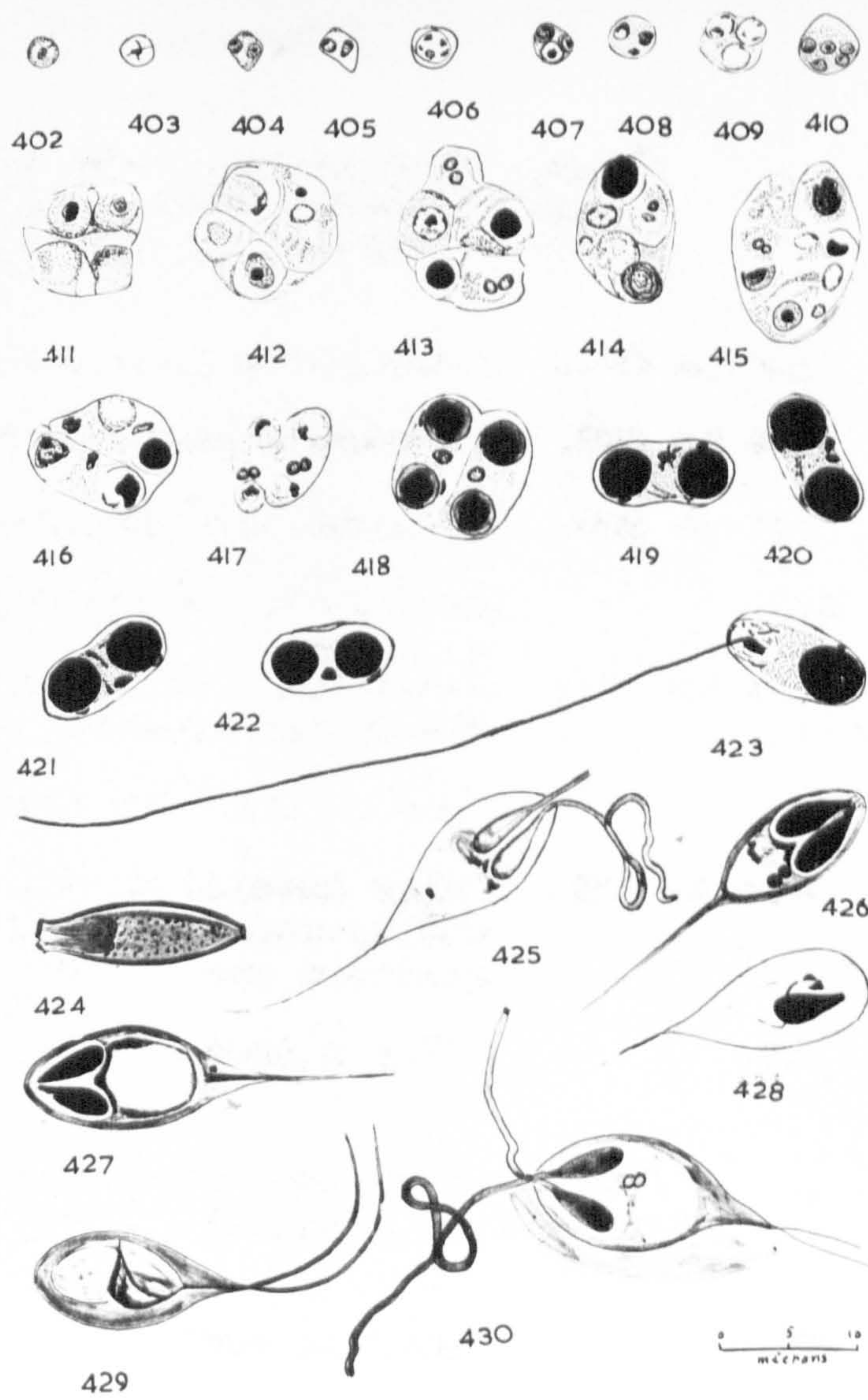




Plate No. XXII.

Henneguya psorospermica from perch. Giemsa.

Figs. 431 to 433      Spores with extruded polar filaments.  
& 436.

Figs. 434 & 435.    Developed spores.

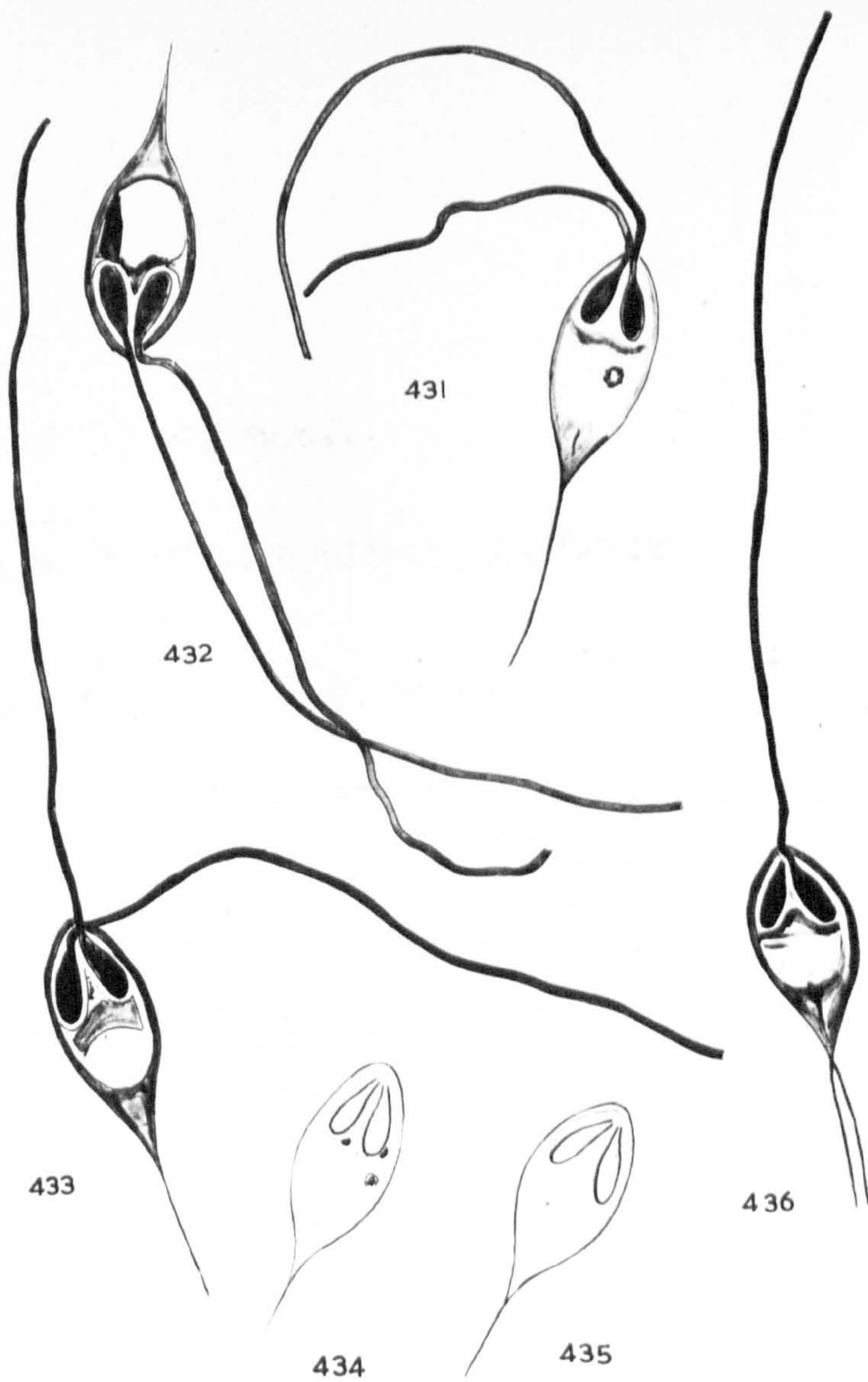


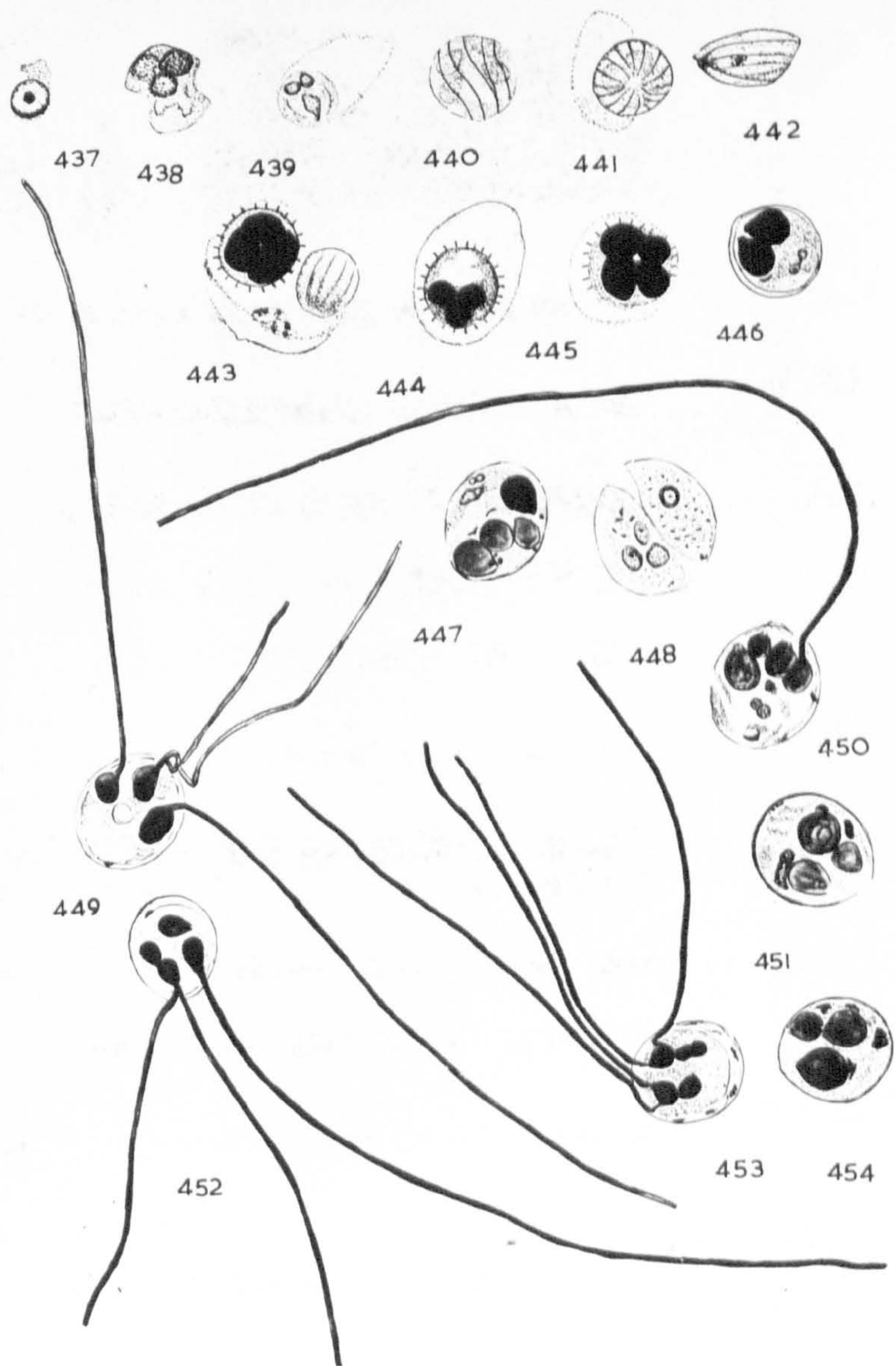


Plate No. XIII.

Chloromyxum phoxini n.sp from  
Phoxinus phoxinus (Minnow). Figures  
from the smear and section of  
gall bladder stained with Giemsa and  
Heidenhain iron haematoxylin stains.

- Fig. 437. Uninucleate pansporoblast with lobopodia.
- Figs. 438,  
439. Trinucleate pansporoblast.
- Fig. 440. Spore showing curved striations.
- Fig. 441. Spores showing striations from front view.
- Fig. 442. The same from side view.
- Fig. 443 to  
445. Spores cog-wheel like.
- Figs. 446 & Fully developed form with two polar  
capsules.
- Fig. 447. The same with four polar capsules.
- Fig. 448. Sporont with two immatured spores.
- Figs. 451 &  
454. Spores with three polar capsules.
- Figs. 449, 450,  
452, 453. Spores with extruded polar filaments.

Plate xxiii





Explanation of Photomicrographs.

- Fig. 455. Trypanosoma striati in the blood of Ophicephalus striatus (X 1600).
- Fig. 456. Slide smear of culture of T. striati from fluid NNN medium modified by Shortt.
- Fig. 457. The same from solid medium (plate).

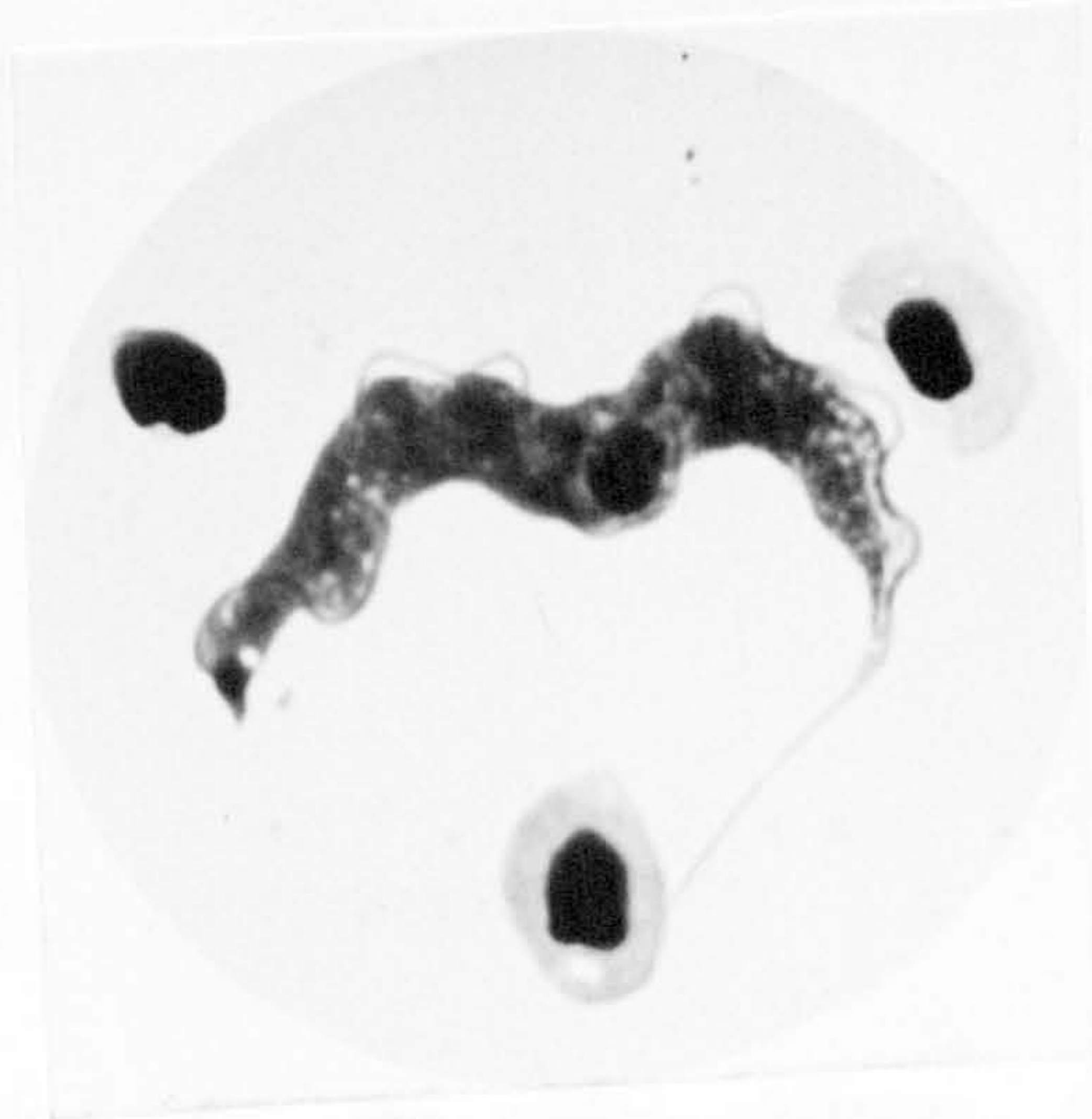


Fig. (455).

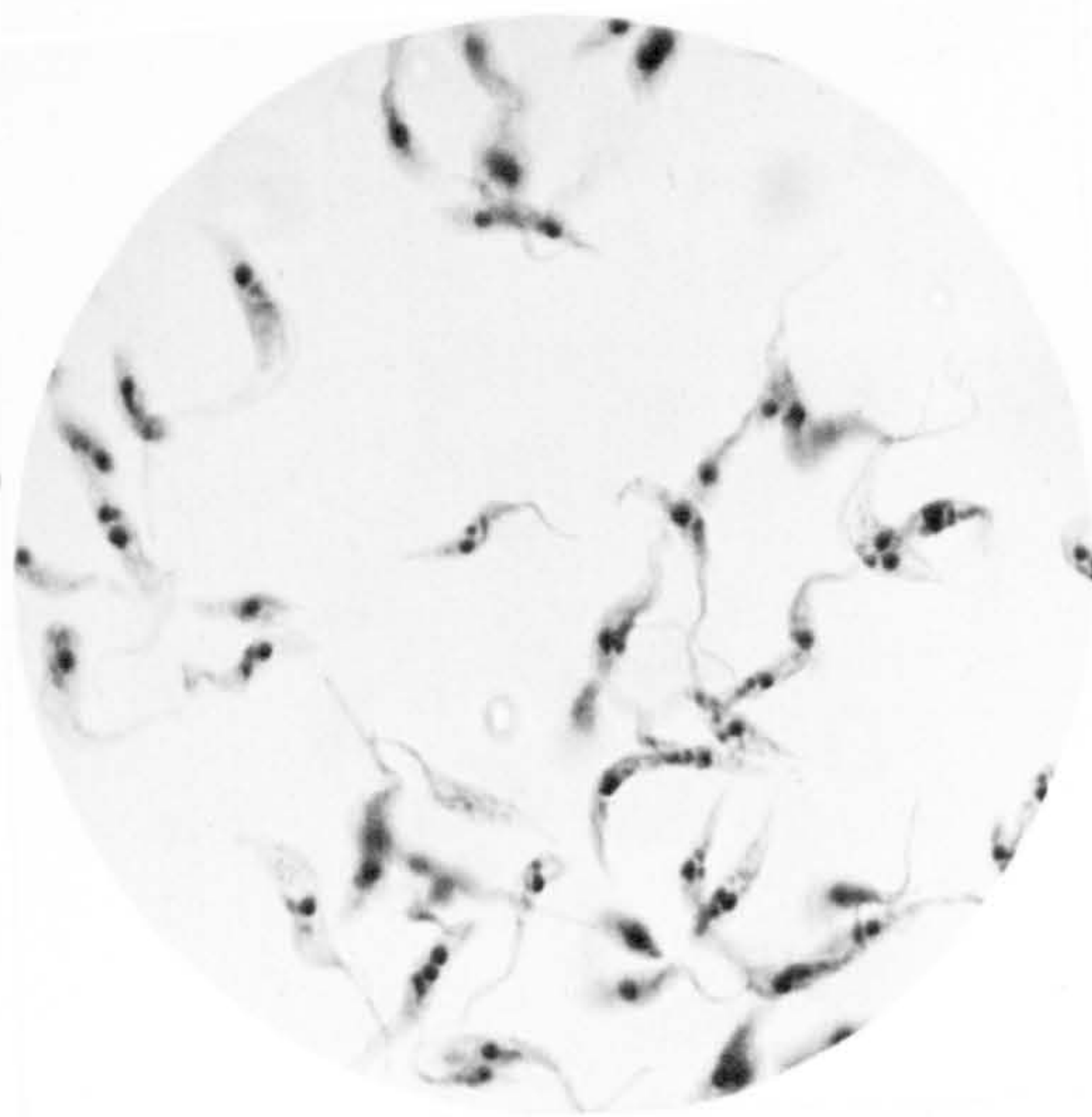


Fig. (456).

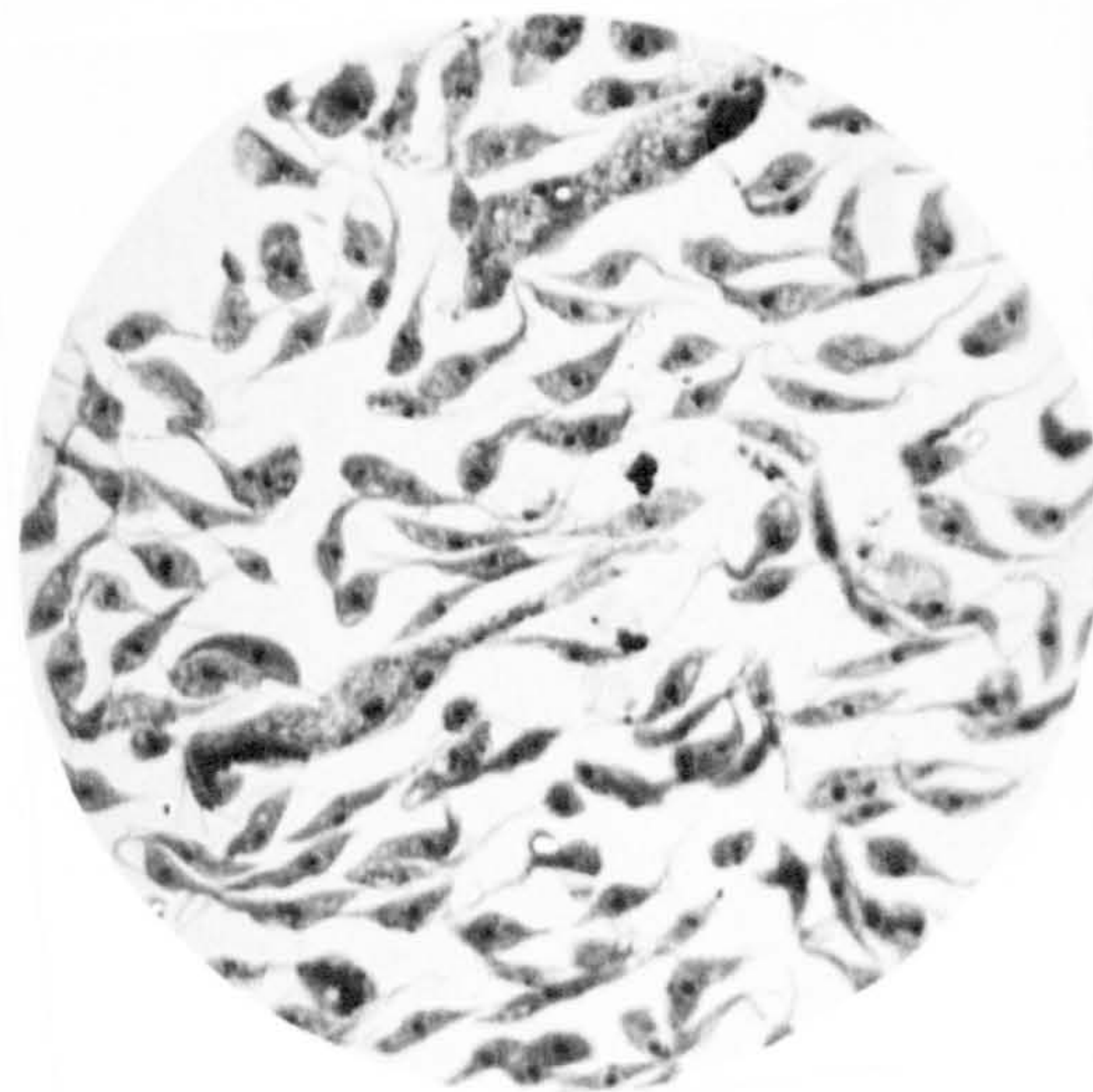


Fig. (457).



Explanation of Photomicrographs.

- Fig. 458. Trypanosoma winchii in the blood of  
Mirror carp (Carp, X 1600).
- Fig. 459. Culture from fluid medium.

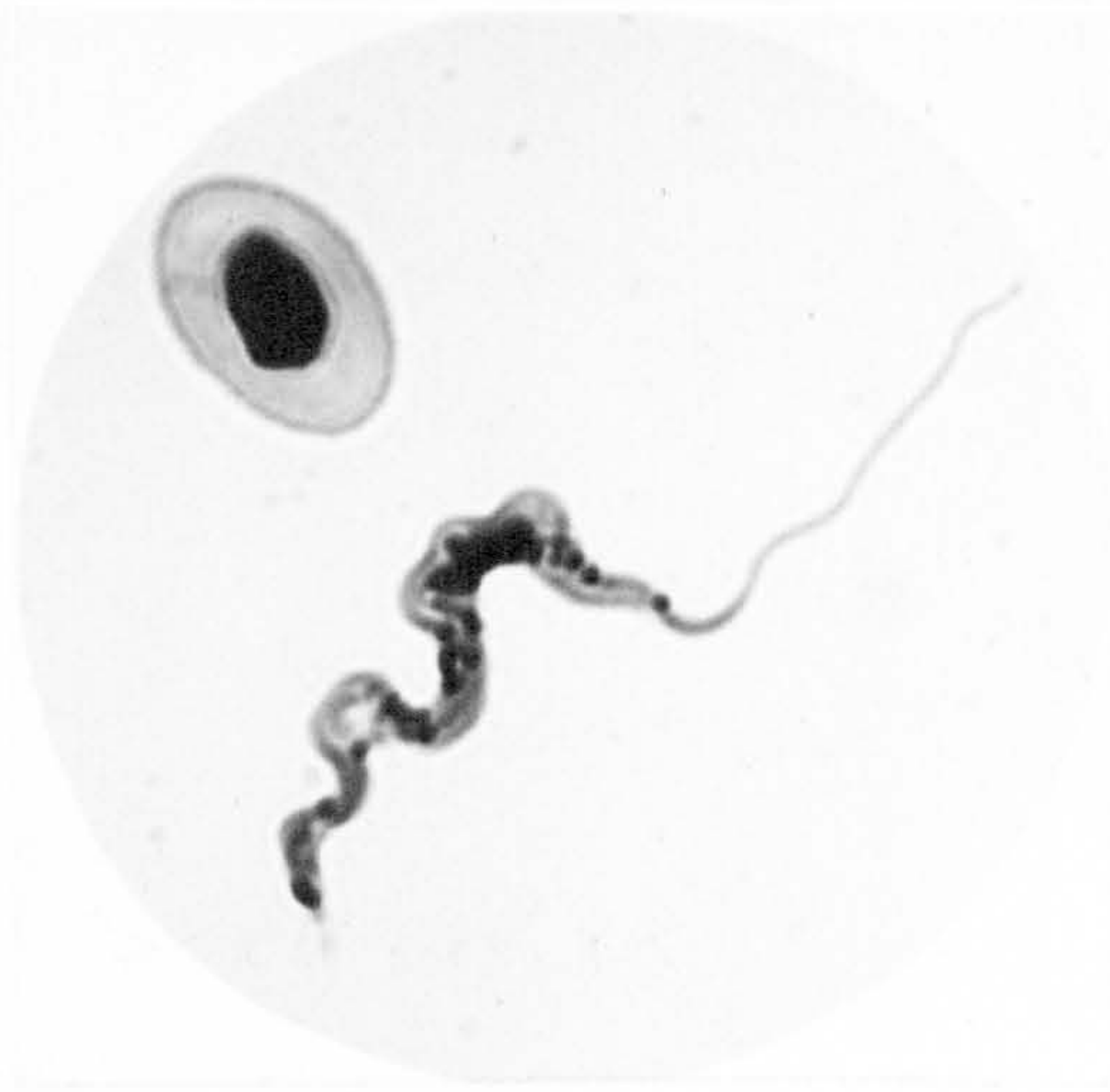


Fig. (458).



Fig. (459).



Explanation of photomicrographs.

Fig. 460. Longitudinal section of leech, Hemiclepsis marginata showing the growth of trypanosomes near the periphery of the crop, six days after feeding.

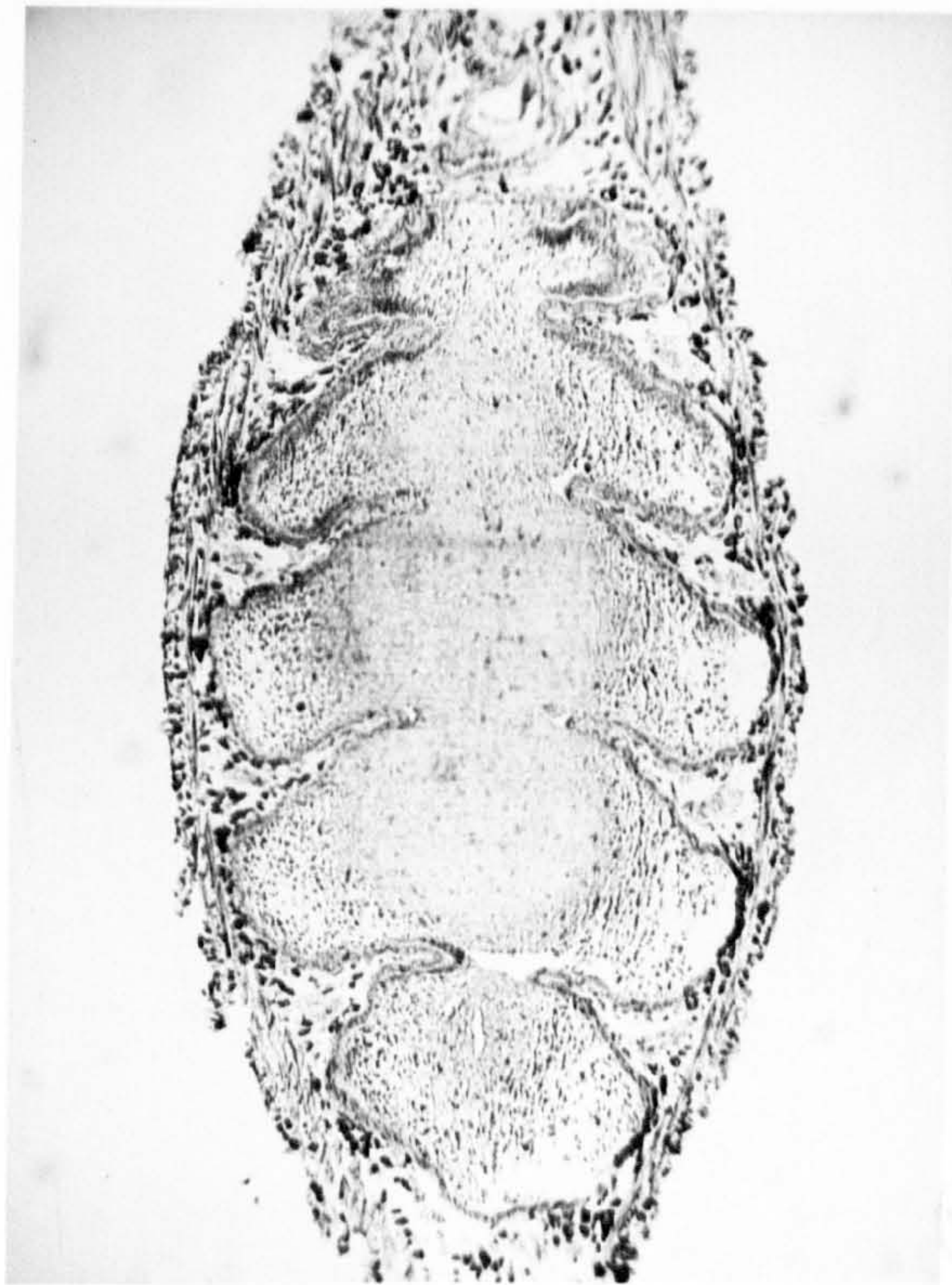


Fig. (460).



### Explanation of Photomicrographs.

- Fig. 461. Longitudinal section of crop of leech,  
Hemiclepsia marginata one day after feeding.
- Fig. 462. The same, three days after feeding.
- Fig. 463. The same, five days after feeding.
- Fig. 464. The same anterior part of the crop, very  
heavy cluster of trypanosomes. Ten days  
after feeding.

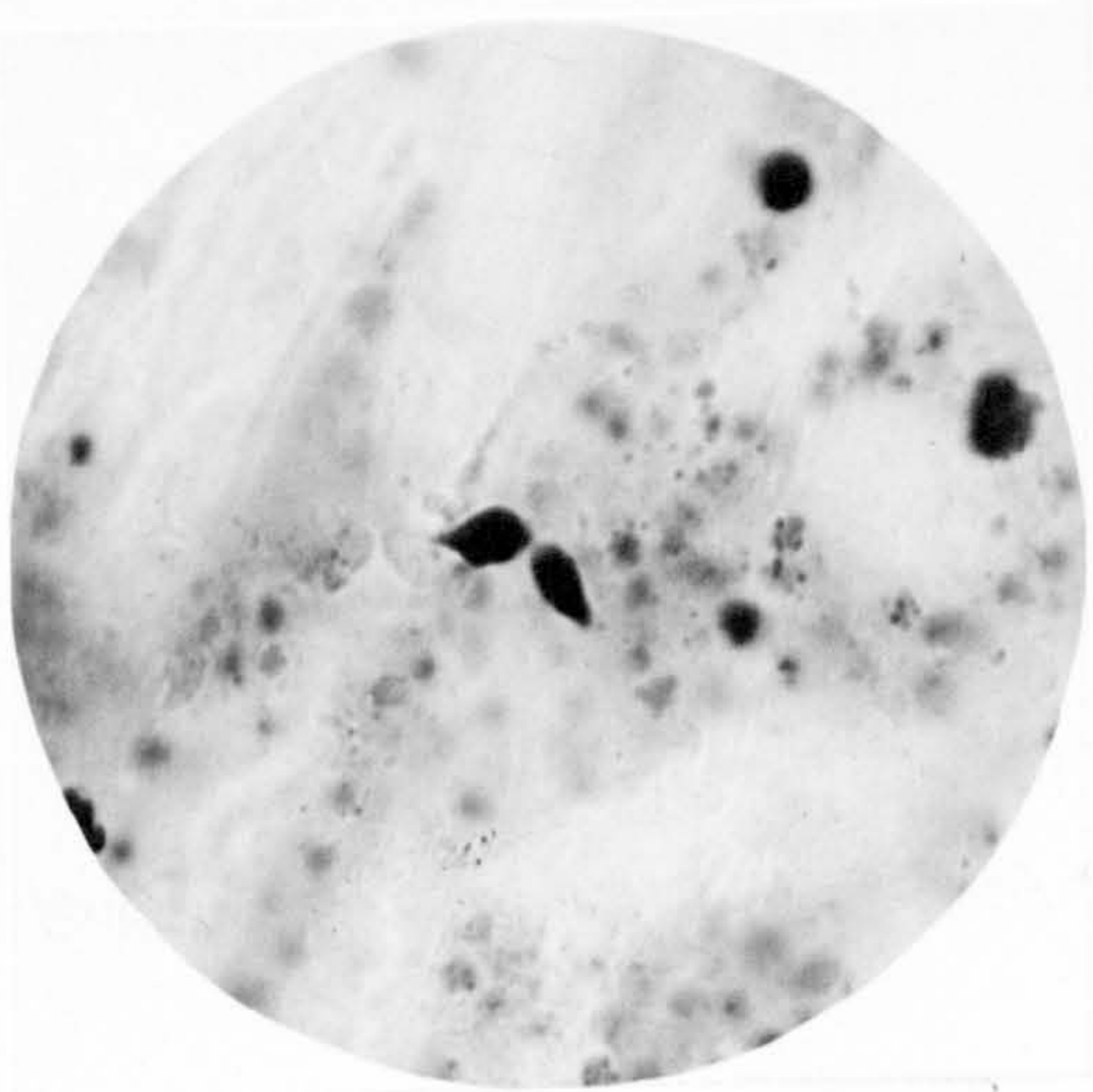


Fig. (461).

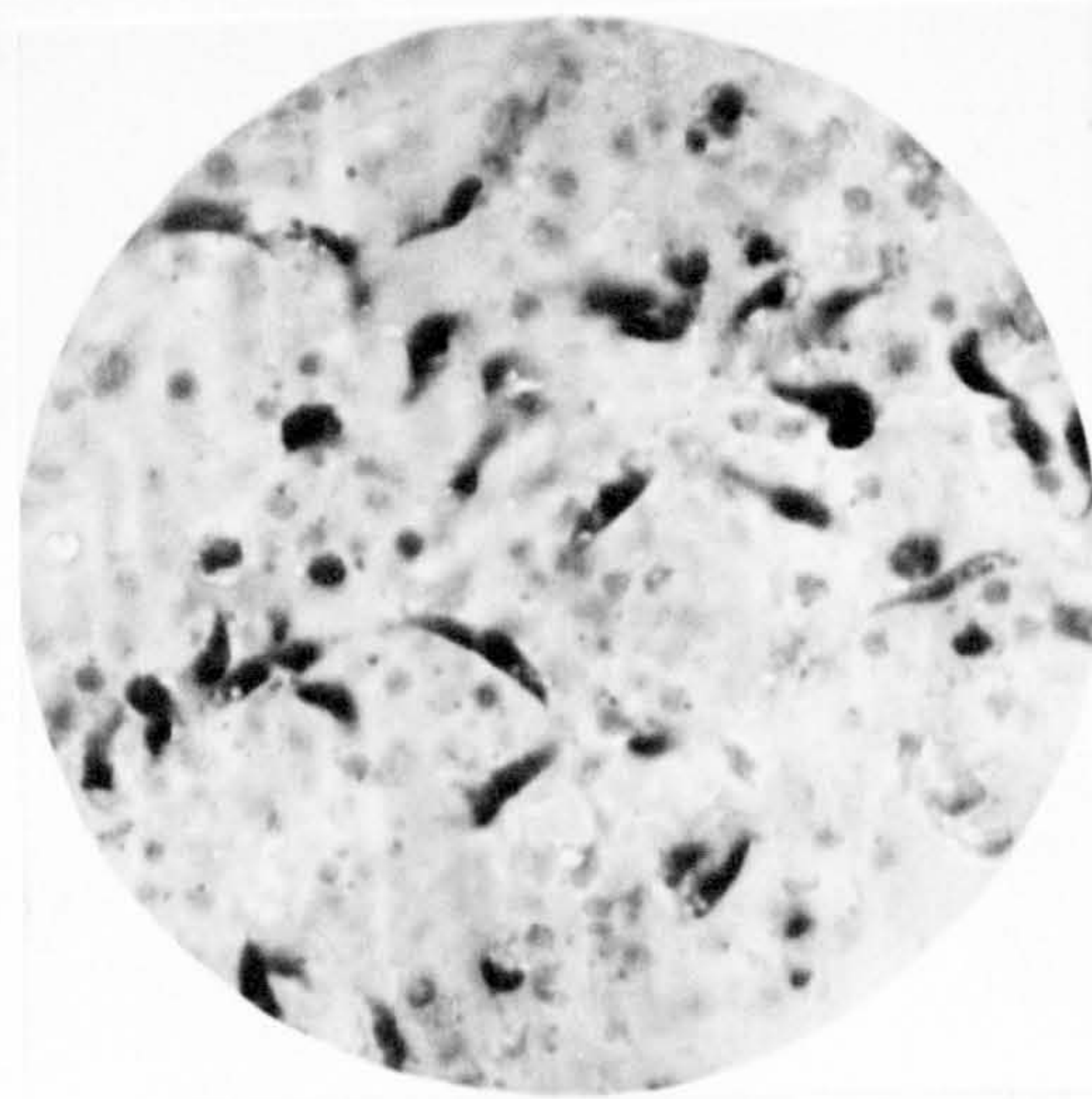


Fig. (462).

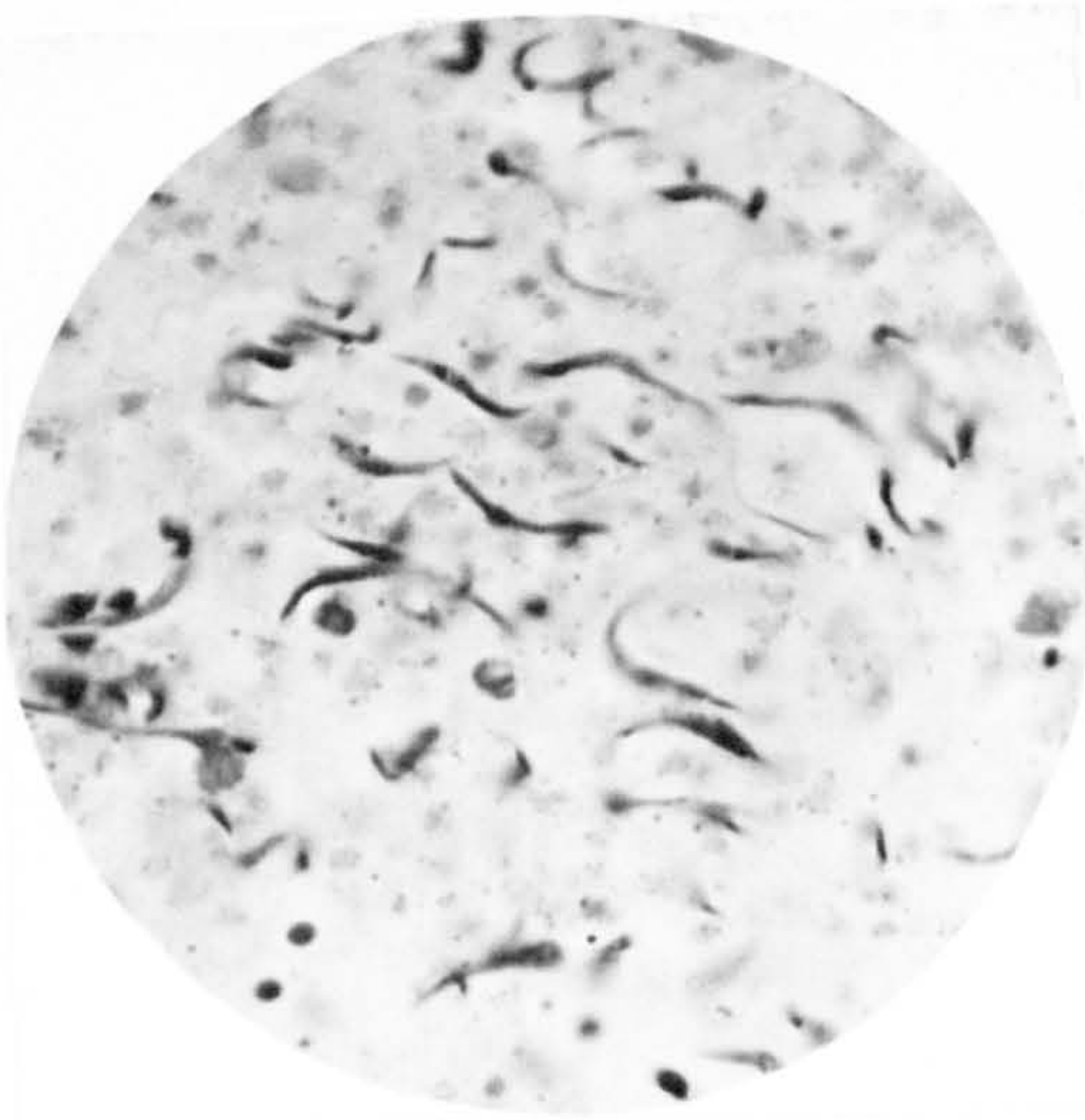


Fig. (463).

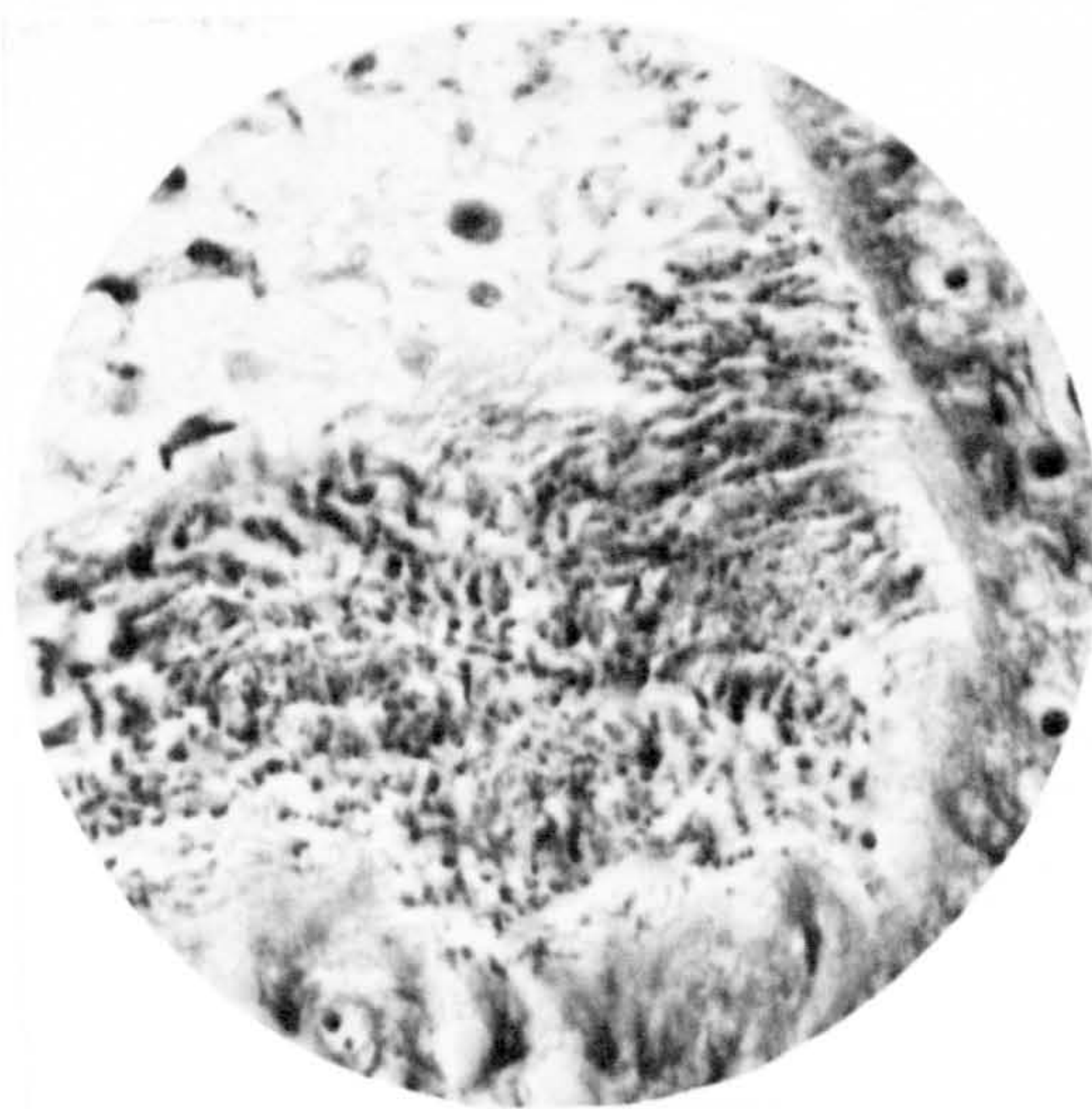


Fig. (464).



Explanation of Photomicrograph.

- Fig. 465. Trypanoplasma willoughbii in the blood of Salvelinus willoughbii (X1600).
- Fig. 466. Smear slide of culture of Tp. willoughbii from fluid NNN medium modified by Shortt. (x1000).

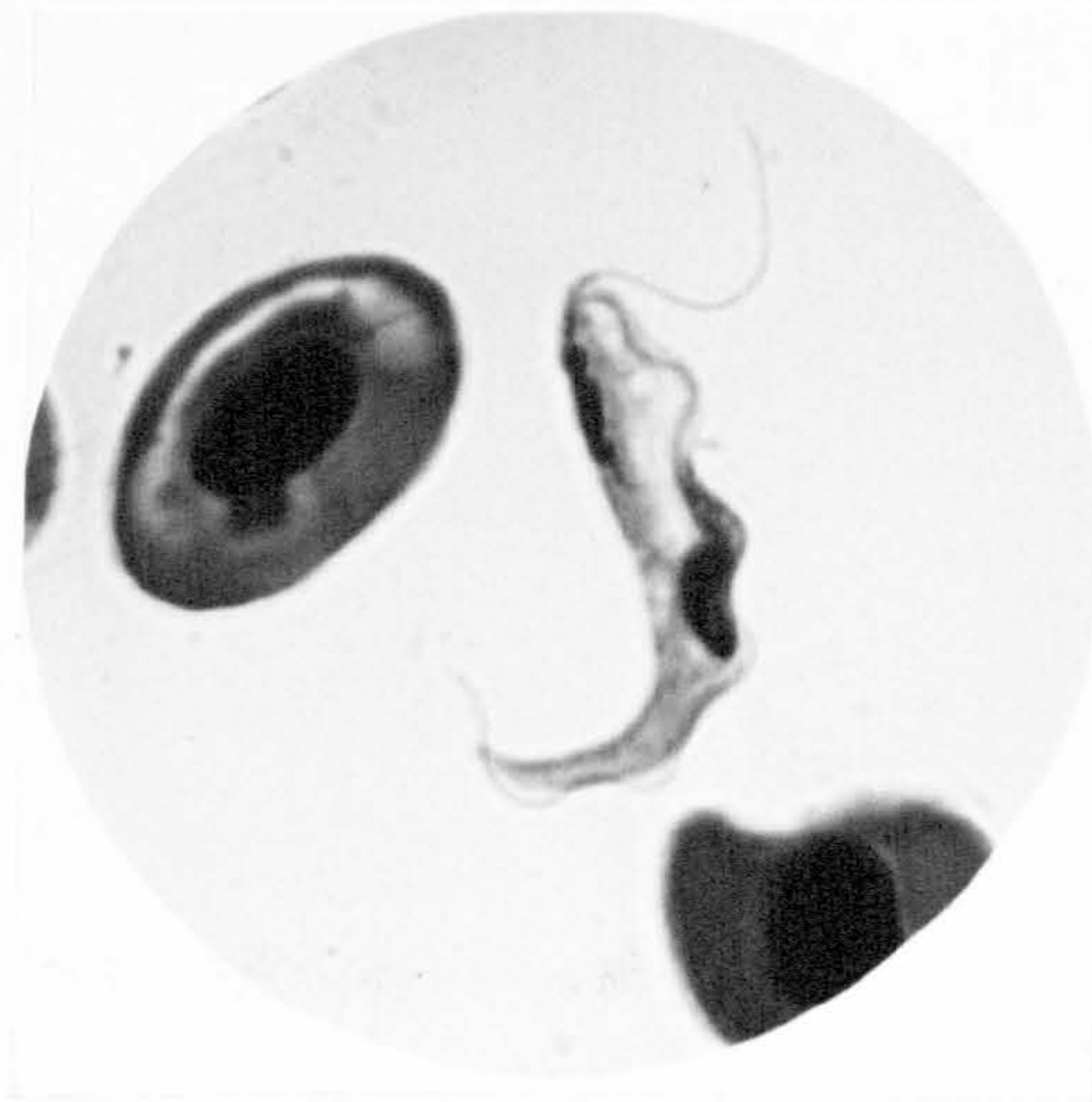


Fig. (465).



Fig. (466).



Explanation of photomicrograph.

Fig. 467. Fish, Clarias batrachus opened up showing nodules of Myxobolus clarii attached to various tissues such as intestine, fat bodies and gonads.





Fig. (467).



#### Explanation of Photomicrographs.

- Fig. 468. Nodules of Myxobolus clarii from various tissues of Clarias batrachus.
- Fig. 469. Myxobolus clarii; smear was made after teasing one of the nodules from the liver. (KOH used for extrusion of polar filaments; Giemsa stain) (x 650).
- Fig. 470. Portion of the liver infected with Myxobolus clarii. (x 200).



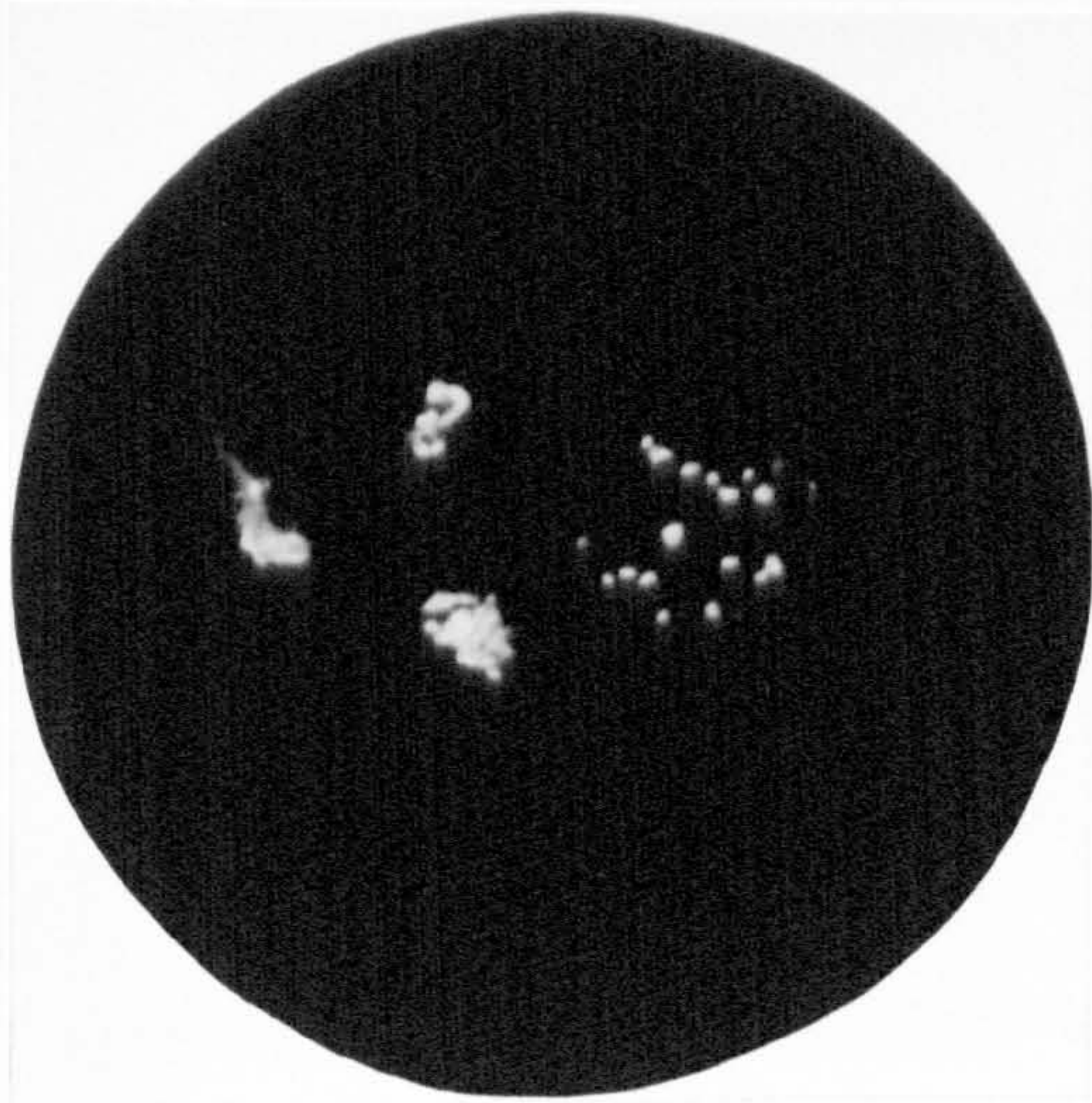


Fig. (468).



Fig. (469).

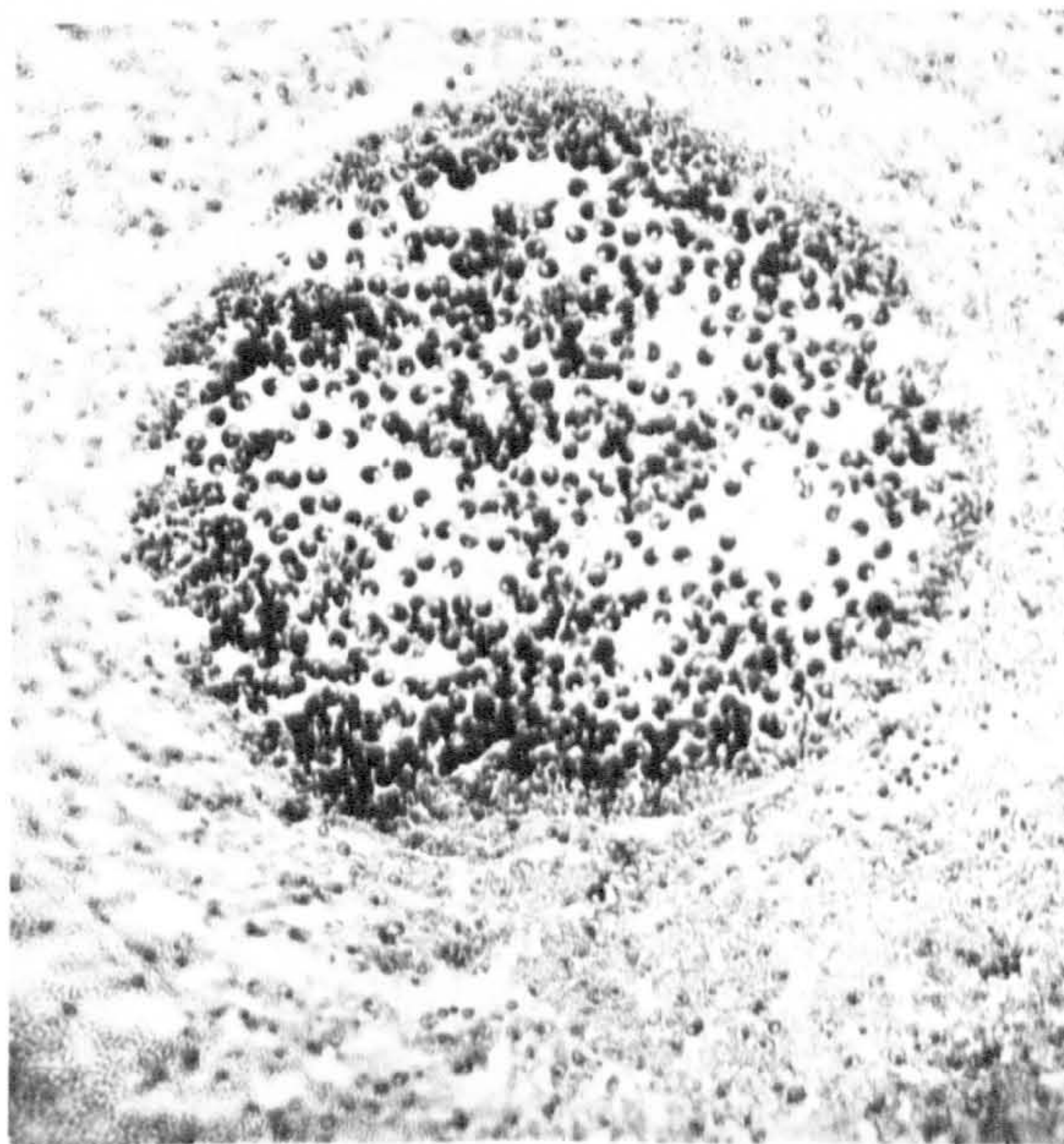


Fig. (470).



Explanation of Photomicrographs.

- Fig. 471. Section of bile duct in liver showing various developmental stages of Zschokkella carpio n.sp. (X 800).
- Fig. 478. Chloromyxum phoxini n.sp. from the bile of Phoxinus phoxinus. (X 1600).

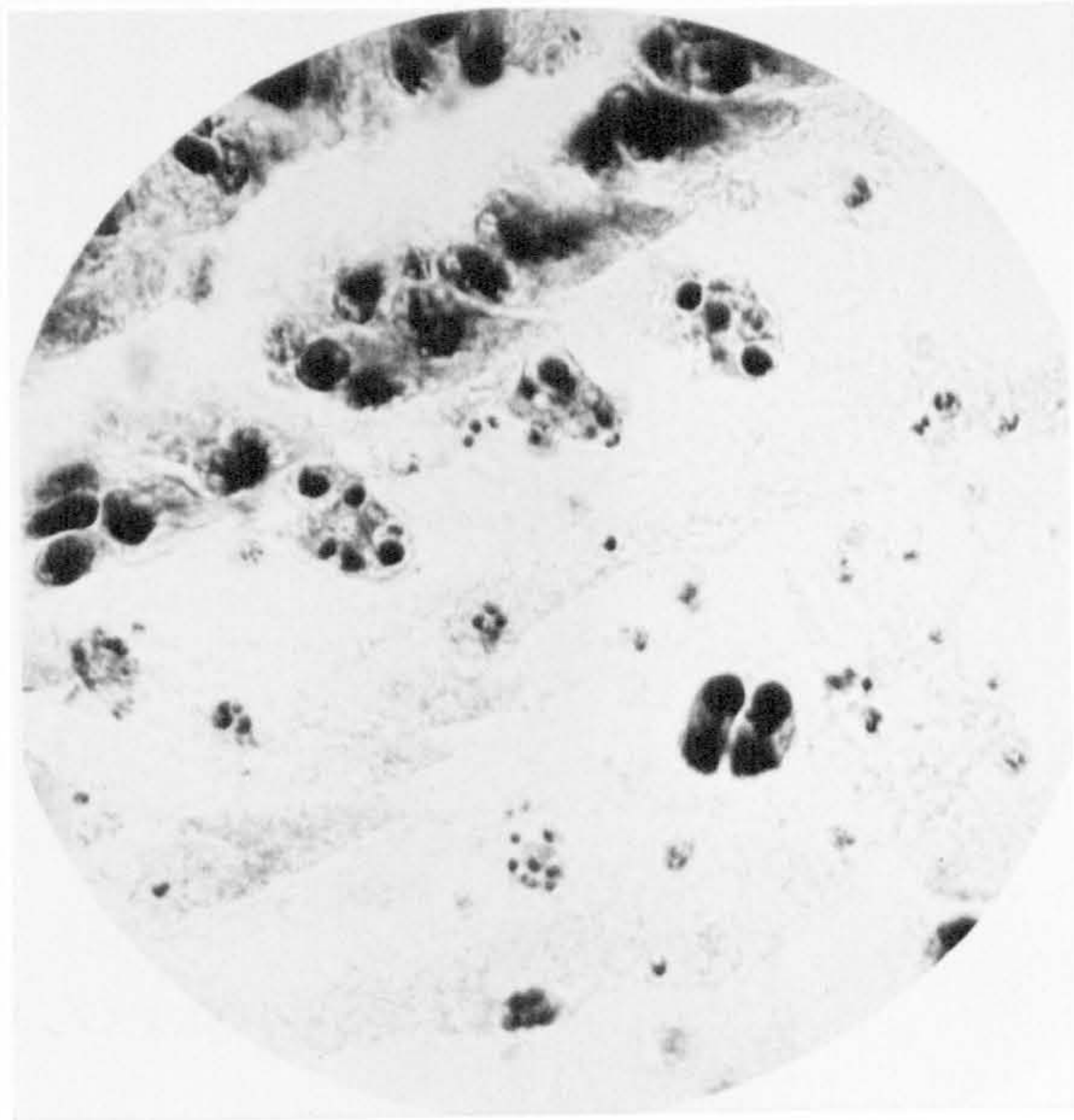


Fig. (471).



Fig. (472).



### Explanation of Photomicrographs.

- Fig. 473. Cyclochaeta domerguii from the gill and scale scraping. (X 800 ) from Cyprinus carpio.
- Fig. 474. Ichthyophtherius multiphilis from the section of muscle of Cottus gobio (bullhead) (X 1600).

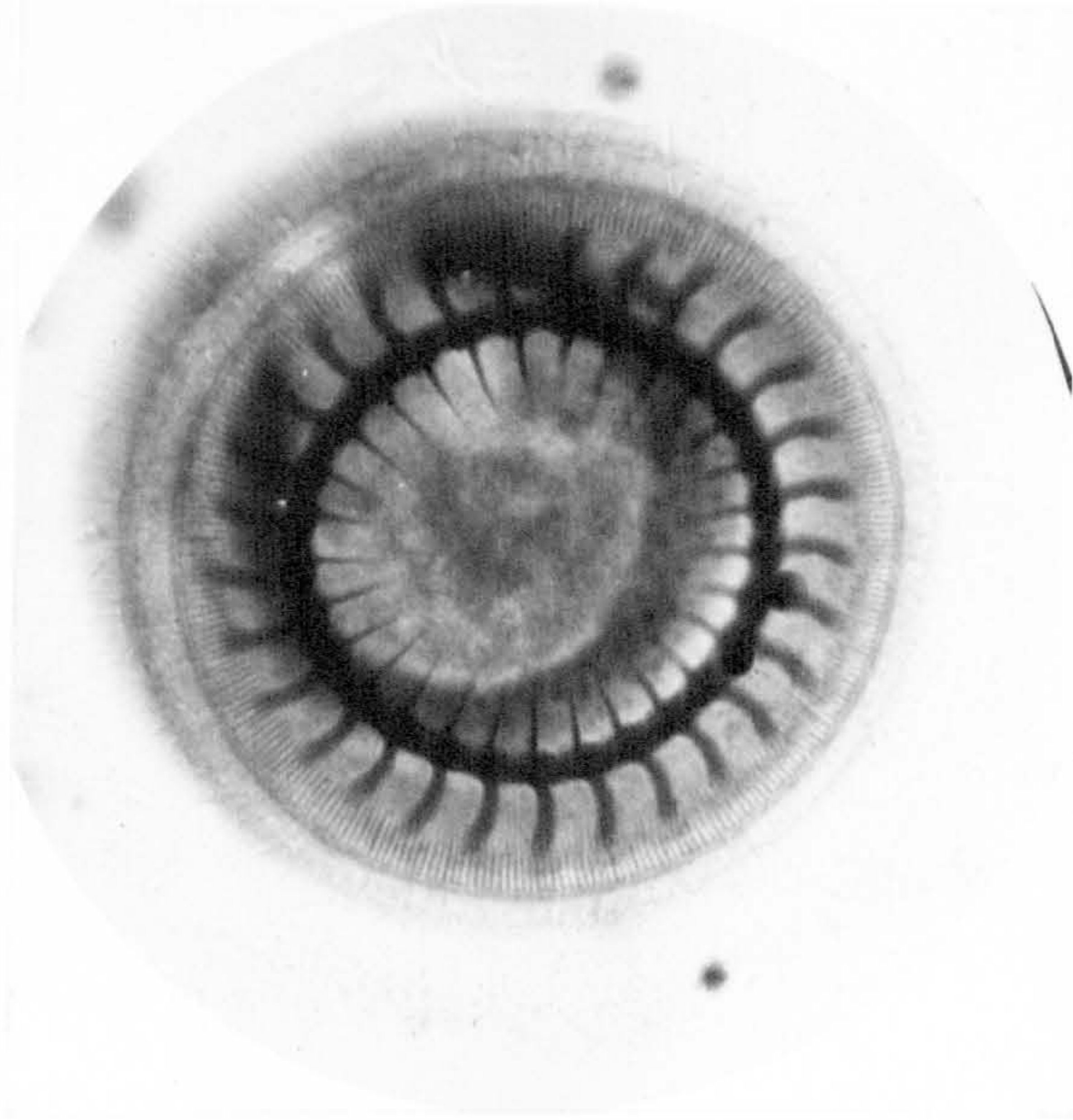


Fig. (473).

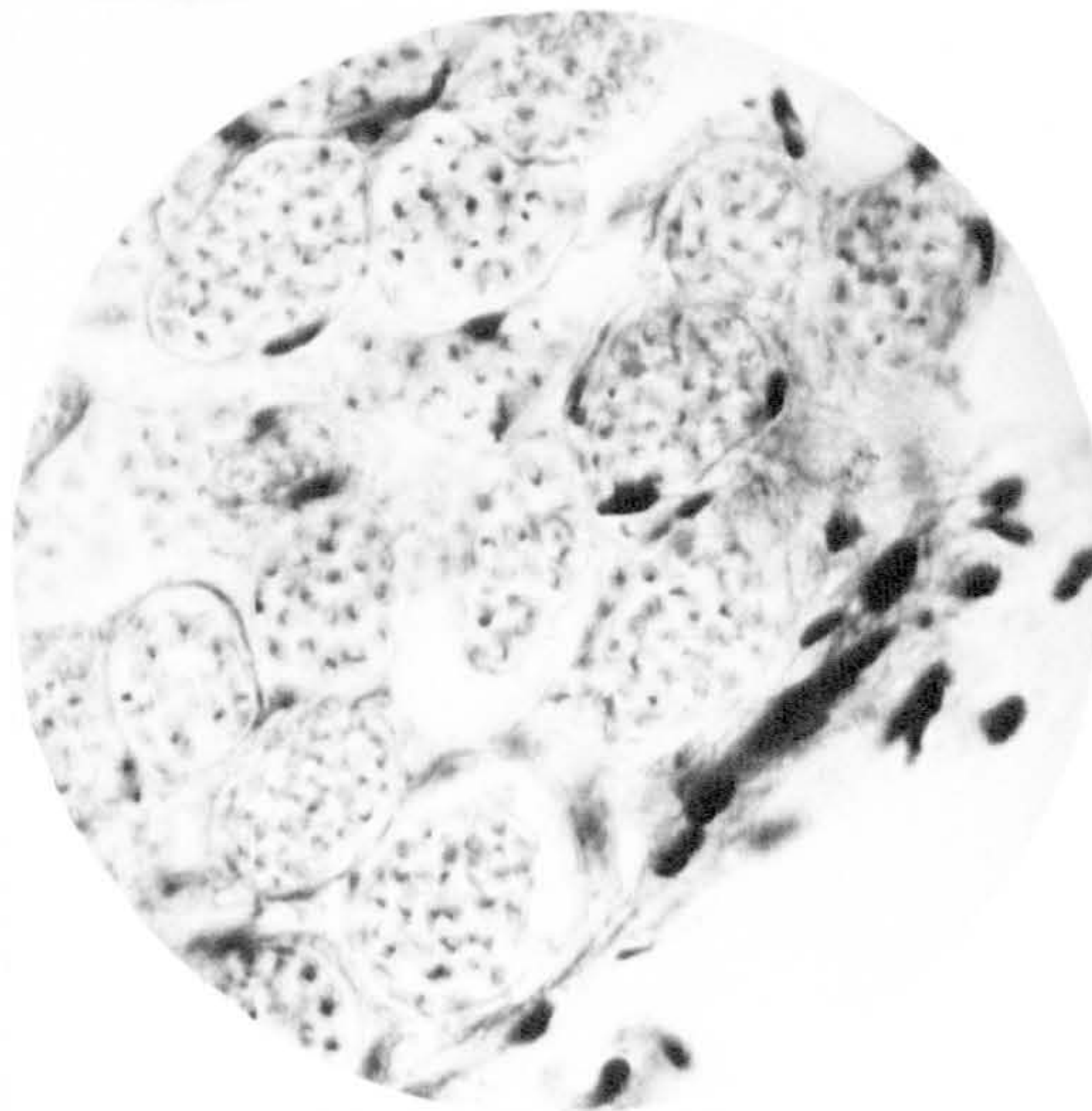


Fig. (474),



ABSTRACT

1. Species of Mastigophora, Myxosporidia and Ciliata (known and new) parasitic in freshwater fish are described and illustrated.
2. Trypanosoma striati from Ophicephalus striatus, an Indian fish, is described. It is polymorphic.
3. Culture of T. striati has been obtained in the medium recommended by Shortt. It has been grown on solid medium (plates) for study of developmental stages, nuclear structure and life history.
4. Trypanosoma winchii n.sp., from the mirror carp has been cultivated in the same medium (fluid and solid) mentioned above to compare the Indian and European forms of this trypanosome.
5. If cultures be induced to grow at temperatures lower and higher than  $24^{\circ}\text{C}$  in the case of the Indian trypanosome and  $15^{\circ}\text{C}$  for the European trypanosome, they proliferate well but the former die at  $34^{\circ}\text{C}$  and the latter at  $26^{\circ}\text{C}$ .
6. The first appearance of trypanosomes in primary cultures from blood takes place in between seven days and one month. When once established, they continue to multiply for an unlimited period after sowing, providing they are sub-cultured every week or fortnight.
7. The trypanosome of Cyprinus carpio when taken with the blood into the crop of a clean Hemiclepsis marginata, starts multiplying after five or six hours. Slender forms are produced 4-5 days later and

become very long, attenuated inoculative forms 6-10 days after being ingested. They then pass forward into the proboscis sheath.

8. Trypanoplasma willoughbii n.sp., has been cultivated and maintained at 15°C temperature in the above mentioned medium. They can grow at a temperature lower than 16°C but die within a week at 18-20°C. They do not multiply on solid medium. The trypanoplasma from culture can be transmitted into another species of fish by means of inoculation with the syringe.
9. T. saccobranchi var. danilewskyi, T. abramis, T. percae, T. guernei and T. cyprini have been encountered.
10. Some species of Myxosporidia - Myxobolus clarii, Zschokkella cyprini, n.sp., Myxidium leucisci n.sp., Henneguya psorospermica and Chloromyxum phoxini, n.sp., have been investigated from Indian and British fish.
11. Two known species of Ciliata - Cylochaeta and Icthyophtherius have been noted from gills, scales and muscles of carp and bullhead.